SUPPLEMENT TO CULTIVATION AND UTILIZATION OF MEDICINAL PLANTS

Edited by
S.S. HANDA & M.K. KAUL

REGIONAL RESEARCH LABORATORY
COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH
JAMMU-TAWI

Production :

Radhe Sham

S.K. Marhkan

D.N. Gupta

Rohini Kaul

R. Neena

Cover Design and art work:

Neeru Vijan

Designed and produced by National Institute of Science Communication, Dr K S Krishnan Marg, New Delhi - 110012 on behalf of the Director, Regional Research Laboratory (CSIR), Jammu - Tawi.

FOREWORD

I am very happy that Prof. S.S. Handa and Dr. M.K. Kaul has prepared a supplement to earlier book on the "Cultivation and Utilisation of Medicinal Plants". This supplement is a timely contribution since all over the world there is great interest now on "green health" products. Medicinal plants are growing in importance day by day because of the wide spread interest in the revitalisation of local health traditions. The goal of "Health for All" by the year 2000 can be achieved only through the adoption of multiple approaches to health care. In such a movement, medicinal plants and the products derived from them have an unique place both in preventive and curative medicine. We therefore owe to Prof. Handa and Dr Kaul a deep debt of gratitude for this labour of love.

M.S. Swaminathan

PREFACE

In 1982, Regional Research Laboratory, Jammu released two companion volumes namely - Cultivation and Utilization of Medicinal Plants and Cultivation and Utilization of Aromatic Plants with the aim of revising and enlarging the original combined book entitled "Cultivation and Utilization of Medicinal and Aromatic Plants" published in 1977. These compendia have served as reference books wherein information on botany, agronomy, chemistry and technology of medicinal and aromatic plants is put together by different specialists. Due to increased demand and significance of the subject matter, these books were reprinted in 1989

During the last decade there has been a flurry of scientific & commerical activities centred around medicinal and aromatic plants. According to a UNDP (1994) report, the annual value of medicinal plants derived from developing countries is about US $ 32 billion (Rs 100, 000 crores).Resurgence of interest in herbal drugs in the western & european countries is evident from the fact that two volumes of British Herbal Pharmacopoeia have been published and $ 33 million worth literature on herbal products was sold in the USA in 1990. The "green wave" in the utilization of medicinal plants resulted in higher consumption. Research workers and industrialists engaged in the field of medicinal plants have increased many folds Considerable efforts are being made all over the world to utilize more and more plant resources for the benefit of mankind. In India extensive research work done on medicinal plants has yielded useful research data. Similarly work on medicinal and aromatic plants is being pursued in developing countries of Asia, Africa and Latin America. Developed countries like Germany, France, USA, Britain and China have shown renewed interest in growing and processing of these plants. UN agencies are recommending, advocating and promoting development of industries based on medicinal and aromatic plants for economic uplift of developing nations. There seems to be a major shift to natural products as these are biologically more compatible and less toxic to human systems as compared to synthetics.

To draw a consolidated thumb-nail sketch of scattered information on all the aspects ranging from field to factory pertaining to medicinal and aromatic plants, a need was felt to publish the supplementary volumes to both the books. The supplements include research papers on important crops not covered earlier, besides

revising and updating articles published in the original two volumes. An honest attempt was made to contact the contributors of the original volumes for revising and updating their research articles. New contributions on topics of current interest were received from different specialists for inclusion in the present books. The format followed in arranging articles in these supplementary volumes is more or less similar to that of the original books which was found to be suitable.

This book on Medicinal plants is aimed at providing a multidisciplinary compendium of information on botany, agronomy, chemistry and technological aspects like post harvest and chemical technology. It covers medicinal plants containing alkaloids, steroids, flavonoids, glycosides, terpenoids, cathartics, adaptogens, antiinflammatories, insecticides, additives and other related active metabolites used in drug and pharmaceutical industries. The book also includes contributions on traditional medicine/ethnomedicine and nomenclatural ambiguity of plants used in Indian systems medicine.

Conservation of diversity, genetic improvement and biotechnological studies on medicinal plants are of considerable significance at present. Articles on these topics have been included. Studies on cultivation and chemistry of some medicinal mushrooms form important and novel contribution. Besides these air borne fungal diseases which create problems in post harvest processing of medicinal plants have been discussed.

The editors had to make some modifications in the presentation of articles to maintain the distinctive flavour of the important reference book on medicinal plants which is supposed to cater the needs of not only professional scientists, technologists & R&D personnels, but also of the laymen, farmers, traders, importers and exporters. However, these modifications and editorial corrections have not changed the inherent structure of articles given by the authors. No work is complete in all respects. Although lot of efforts have been put to exclude ommissions, yet there could be some. The editors hope that the useful material provided in each article will go a long way to help people working on different aspects of medicinal plants.

We are extremely indebted to Dr. R S Kapil for initiating the work. All the authors who have contributed their articles were prompt in replying our queries. Thanks are due to Ms Rekha for typing the manuscript. Ms Kiran Kaul, Miss Suman Chib assisted in proof reading whereas Miss Uma Jamwal helped in drawing work. We are thankful to Dr. G P. Phondke, Director, National Institute of Science Communication (CSIR), New Delhi and his team of dedicated workers for printing & production of this work.

Regional Research Laboratory, Jammu - 180 001

S.S Handa
M K Kaul

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Therapeutic Potential of Medicinal Plants: A Global Perspective

Ashok D.B. Vaidya

CIBA Research Centre
Bombay 400 063

It is shocking to learn that out of 250,000 may be 500,000 higher plants on the planet earth, around 50-60% will be destroyed in ten years[2]. So the time is running out for many scientists, across several disciplines, to act and act now. There is a need to prioritize and put in the best collective efforts to select out the plants with significant health potential. It was with this urgency that a team of technical experts gathered often to discuss and decide on 111 selected Indian Medicinal Plants. The monograph on these plants, from India, has been well-received nationally and internationally[3]. Table 1 shows the list of the 111 medicinal plants as selected. Small monographs of such a nature have also been published from China, Vietnam and other countries. The emphasis is more for the use of the parts of the plant, as formulated in the respective traditional system of medicine rather than on the less productive and long route of isolation of the active principles. The approaches are not mutually exclusive. But to ensure the biodiversity of plants, standardized preparations as therapeutic agents should take precedence over the long (10-12 year) period of a new (active principles) drug development[4].

In India, we often wake up to our own therapeutic wisdom only after a recognition comes from the west This was the fate of many plant-based leads and the state-of-affairs continues to be the same. It was in 1931 that Sen and Bose had urged, in their epoch-making paper[5], medical men all over the world to try *Rauwolfia serpentina* and see for themselves if it were into "a drug of rare merit" The fervent appeal was lost in the wilderness for more than a couple of decades[6].

Table 1 - III Indian Medicinal Plants for the Monograph

Acacia arabica	*Cyperus rotundus*	*Plantago ovata*
Acacia catechu	*Dolichos biflorus*	*Pluchea lanceolata*
Achyranthes aspera	*Eclipta alba*	*Psoralea corylifolia*
Aconitum heterophyllum	*Elettaria cardamomum*	*Pterocarpus marsupium*
Adhatoda vasica	*Embelia ribes*	*Pterocarpus santalinus*
Aegle marmelos	*Enicostemma littorale*	*Punica granatum*
Allium sativum	*Eugenia aromatica*	*Raphanus sativus*
Aloe indica	*Evolvulus alsinoides*	*Ricinus communis*
Alpinia galanga	*Ferula foetida*	*Rubia cordifolia*
Alstonia scholaris	*Ficus bengalensis*	*Santalum album*
Andrographis paniculata	*Ficus racemosa*	*Saraca indica*
Asparagus adscendens	*Ficus religiosa*	*Saussurea lappa*
Asparagus racemosus	*Fumaria officinalis*	*Sida cordifolia*
Balasamodendron myrrha	*Glycyrrhiza glabra*	*Smilax chinensis*
Bambusa arundinacea	*Hedychium spicatum*	*Solanum indicum*
Bergenia ligulata	*Hemidesmus indicus*	*Solanum nigrum*
Boerhaavia diffusa	*Hibiscus rosa-sinensis*	*Solanum xanthocarpum*
Bombax malabaricum	*Holarrhena antidysenterica*	*Strychnos nux-vomica*
Boswellia serrata	*Hyoscyamus niger*	*Swertia chirata*
Butea frondosa	*Inula racemosa Hook*	*Symplocos racemosa*
Carum copticum	*Ipomoea digitata*	*Tephrosea purpurea*
Cassia angustifolia	*Lawsonia inermis*	*Terminalia arjuna*
Cassia fistula	*Leptadenia reticulata*	*Terminalia belerica*
Celastrus paniculatus	*Melia azadirachta*	*Terminalia chebula*
Centella asiatica	*Mentha arvensis*	*Tinospora cordifolia*
Cichorium endivia	*Mesua ferrea*	*Tribulus terrestris*
Cinnamomum tamala	*Mimosa pudica*	*Trichosanthes dioica*
Cissus quadrangularis	*Mucuna pruriens*	*Trigonella foenum-graecum*
Citrus medica	*Nardostachys jatamansi*	*Tylophora indica*
Clerodendron serratum	*Nelumbo nucifera*	*Valeriana wallichii*
Commiphora mukul	*Ocimum sanctum*	*Vanda roxburghii*
Crataeva nurvala	*Operculina turpethum*	*Viola odorata*
Crocus sativus	*Phyllanthus emblica*	*Vitex negundo*
Cuminum cyminum	*Phyllanthus niruri*	*Wedelia calandulacea*
Curculigo orchioides	*Picrorhiza kurroa*	*Withania somnifera*

Table 2 lists the names of plants, which have showed promise in India, by clinical use and experimental data, but were recognised much later or are yet to be recognised. One of the reasons is that the early publications are viewed as 'prior art' and patents are hard to come by and defend. Hence major pharmaceutical industries, with dominant profit incentive, do not take any interest[7].

Table 2: Medicinal Plants with Therapeutic Promise

Rauwolfia serpentina	Hypertension, anxiety
Picrorhiza kurroa	Hepatitis, asthma
Commiphora mukul	Hyperlipidemia
Psoralea corylifolia	Leucoderma, psoriasis
Mucuna pruriens	Parkinson's disease
Tinospora cordifolia	Hepatoprotection
Piper longum	Recurrent infections
Curcuma longa	Cancer prevention
Embelia ribes	Parasitic infections
Azadirachta indica	Eczema
Phyllanthus amarus	Hepatoprotective
Phyllanthus emblica	Anti-aging
Withania somnifera	Anti-stress
Ocimum sanctum	Adaptogen

There are currently no guidelines for the cultural property rights of a nation or a region over their therapeutic wisdom of many centuries. There has to be an international debate and decisions on such guidelines[8]. The Dunkel Draft and all the loud champions of the intellectual property rights do not pay even lip-service to a nation's traditional health knowledge[9]. I believe that as the air we breathe or the water we drink cannot be and should not be patented, the natural products, in their crude and standardised form are a global heritage to be shared for the relief of human suffering. Only human inventiveness of new molecules derived from the nature's molecules, which undergoes a long and expensive period of drug development of 10-12 years, deserves a return on investment by intellectual property rights[10]. These ideas may sound unusual but if not accepted the extinction of plants of therapeutic and commercial interest will be even faster; the health wisdom of centuries will vanish soon.

For a discussion, a vision and a set of values may be tentatively proposed. The vision about the vast therapeutic potential of plants has to include five dimensions of values: 1) relief of human suffering should take precedence over market-exclusivity,2) ecosystem-salvage and maintenance of biodiversity should be ensured before a large-scale collection of the plant species, 3) regional, national or cultural property rights of therapeutic knowledge to be respected and financially compen-

sated, 4) the hierarchial position of the plant use, amongst the available therapeutic options, be defined by consensus meetings, 5) appropriate resource-allocation for an interdisciplinary research, for a fast-track development, to be ensured. In view of the threat of a rapid extinction of higher plants, it is urgently needed that W.H.O. and other international agencies including pharma-industry convene an early meeting of experts to evolve feasible guidelines and global control mechanisms based on the aforesaid or related values.

The current market for the health-care natural products, in U.S.A., is approximately around $ 15 billion. The global projections are difficult to arrive at but essentially involve huge sums of money. Hence it is desirable that social accountability is established for a sustained biodiversity. The green movement and the environmental battle cry are social forces to be harnessed for safeguarding the vast therapeutic potential of medicinal plants. But also as Potrykus put it, just a promise of finding something for AIDS or cancer is not a strong argument for the preservation of rain forests. It is a must for our survival[illegible].

Certain major health problems, responsible for large scale morbidity and mortality, have to be shortlisted. Situations, where preventive or therapeutic modalities are inadequate or absent, demand concerted research efforts, at multiple levels of biological organisation, by a transdisciplinary approach[1,13,14,illegible]. A list of global scourges is short listed in Table 3. Medicinal plants with therapeutic potential for such diseases need to be investigated extensively and urgently.

A major need is for different types of immuno potentiating agents which can be useful in enhancing humoral or cellular immunity by acting at different stage of the immune cascade. *Asparagus racemosus*[17], *Embelia ribes*[17], *Piper longum*[illegible], and *Ocimum sanctum*[9], require close research attention by immuno-pharma-

Table 3. A short List of Indications

1	Immune suppression
2	Cancer/Cell transformation
3	Stroke and Coronary artery disease
4	Arthritis/Rheumatic disorder
5	Hepatitis and liver damage
6	Respiratory allergy
7	Atopic dermatitis and urticaria
8	Diabetes mellitus
9	Memory and learning disorders
10	Menorrhagia
11	Viral infections
12	Aging disorders

cologists; oral history, clinical use-experience and early experimental data already suggest such a potential.

Notwithstanding a massive and relatively unproductive screening programme by the National Cancer Institute (USA) for cytotoxicity for cancer cells of natural products, the success of *Vinca* alkaloids and Taxol^R suggest that there may be several such active principles with a wider therapeutic margin. A scrutiny of unusual responses, in patients with advanced cancer using alternative systems, has possibilities of detecting plants with therapeutic potential[21]. Recently there has been an upsurge of interest in the antimutagenic activity of *Curcuma longa*, β- carotene-containing plants, etc. Scavenging of free radicals is involved in protection from mutagenic insults. It is worth-while to develop simple, and reproducible models for a rapid screening of free-radical scavengers. Such compounds can also show promise against ischaemic damage to heart, brain and other tissues. *Picrorhiza kurroa* and Arogyawardhani are shown to have hepatoprotective effect in animal models and clinically useful in viral hepatitis[22,23]. The active principles of the plant Viz picrocides - I and II inhibited the lipid peroxidation caused by CCl_4 in isolated hepatic microsomes. Lipid peroxidation can be studied in blood cells or other cells grown in tissue culture. Such *in vitro* models have to be properly correlated to *in vivo* situations, based on the dosage, kinetics, metabolism, etc.[24]

Atherosclerosis and ischemic myocardial or brain damage are major causes of morbidity and mortality. There are several plants, which when coupled with a healthy life style, can be of preventive and therapeutic use in the susceptible and high risk groups of patients. Table 4 shows a list of such plants. *Commiphora mukul*[25] or *Boswellia serrata*[26], *Garcinia cambogia*[27,28], *Allium sativum*[29], *Terminalia arjuna*[30,31], *Curcuma longa*[32] and *Terminalia chebula*[33] may offer excellent potential for different subsets of patients with hyperlipidaemia and atherosclerosis. There has to be greater emphasis on the required increase in HDL and a reduction in LDL and triglycerides, besides objective studies showing a regression in the atheromatous placques. Invasive angiographic techniques in man will have to be replaced by better non-invasive techniques to permit ethically justified clinical trials in man[34]. But experimental studies in reversal of athero sclerotic placques should precede the clinical studies. The phase 1 and Phase 2 studies in volunteers and patients are desirable to assess the safe dosage range, clinical tolerability and organ- function safety profiles[35,36]. Large scale use-experience trials, in Indian medicine, of cardioactive drugs are also possible. But proper planning and management are needed for reliable data[37].

Arthritis and rheumatic disorders constitute a major cause of morbidity. The current therapeutic armamentarium is not often adequate and for chronic diseases, the side-effects are sometimes non-acceptable. Yogaraj-guggulu offers a good choice for the management of rheumatoid arthritis. Phase I studies showed good tolerability of even large doses[38]. Other plants which offer a potential are :*Boswellia serrata*[26], *Commiphora mukul*[25], *Pluchea lanceolata*[39], *Ricinus communis*[41],

Table 4. Plants for Atherosclerosis and Ischaemic Disorders

1	*Commiphora mukul*
2	*Boswellia serrata*
3	*Garcinia cambogia*
4	*Allium sativum*
5	*Terminalia arjuna*
6	*Curcuma longa*
7	*Terminalia chebula*
8	*Allium cepa*
9	*Plantago ovata*

Morus alba[40], *Pandana foetida*[4], *Vanda roxburghii*[43], *Glycyrrhiza glabra*[44,45] *Curcuma longa*[33], *Ficus religiosa*[46], *Boerhaavia diffusa*[47], *Coptis chinensis*[48], *Berberis aristata*[49,50], etc. Only some of these have been studied clinically but there is a need to look at the specific subsets or rheumatoid arthritis. There is relatively a paucity of compounds/plants for osteoarthritis. This area can be investigated more objectively with the modern imaging techniques and even arthroscopy. Preventive modalities can be extremely desirable for osteoarthritis of hips and knees.

Hepatoprotective medicinal plants have been quite extensively investigated experimentally but clinical trials in hepatitis or liver cirrhosis are not easy to carry out. Even then the following plants have shown promise: *Phyllanthus amarus*[51,52], *Picrorhiza kurroa*[23], *Andrographis paniculata*[53,54], *Zingiber officinale*[55], *Alangium chinense*[56], *Piper longum*[57], *Piper nigrum*[58], *Ricinus communis*[41], *Eclipta alba*[59], *Wedelia calandulaceae*[60], *Boerhaavia diffusa*[47], *Tephrosia purpurea*[61], etc. But sometimes tall claims are made, for example a disappearance of hepatitis B virus, which could not be confirmed by later studies[52]. Drugs, alcohol, chemicals and environmental pollutants are known to induce liver damage. Medicinal plants which can prevent or relieve such a damage are clinically needed. But the rigour of clinical design and statistical analysis are prerequisites.

Bronchial asthma and upper respiratory tract allergy causes a sizeable morbidity and even some mortality. Currently topical steroids, antihistaminics, mast cell membrane stabilisers, adrenergic α- and β1-agonists and new cholinergic antagonists are employed. But there is a need for compounds and medicinal plants which are preventive in action or reduce the need of other drugs by making the severe attacks of asthma milder, over a period. Several medicinal plants have been used. Sri Raman Maharshi used to advise *Picrorhiza kurroa* as a long term preventive modality[23]. In children, *Piper longum* has been studied and frequently recommended[57]. As the major anti-asthma plant drug was a world contribution from China - Ma Huang or *Ephedra sinensis*, we need to look at Chinese plants used for nasal allergy and asthma. *Zingiber officinale*[55], *Anisodus tanguticus*[62], *Ardisia*

japonica[63], *Aristolochia contorta*[64], *Asarum* spp[65], are some of such plants listed in a monograph on Chinese drugs of plant origin. These plants have to be investigated further alongwith the other Indian plants like *Tylophora indica*[66], *Solanun indicum*[67], etc. For asthma Ayurveda also prescribes *Shodhana Chikitsa*. Currently Sharadini Dahanukar's unit at K.E.M. Hospital (Bombay) has initiated work in this direction. Such complementarity of non-drug and drug modalities is well-known in the traditional systems of medicine and has to be understood in terms of the pathophysiology of the disease.

Allergy and urticaria constitute a sizeable work load in dermatology. Contact dermatitis and eczema also have a high incidence. Modern therapy with potent topical corticosteroids and systemic antihistaminics are used alongwith avoidance of the specific allergens. Human pharmacological methods to study effects of the medicinal plants on histamine-induced flare and wheal can be gainfully employed in clinical research in volunteers and patients. Many plants have been claimed and used for skin allergy or eczema. Some of the plants offering therapeutic potential are - *Curcuma longa*[32], *Berberis aristata*[49], *Rubia cordifolia*[69], *Azadirachta indica*[70], *Embelia ribes*[71], *Glycyrrhiza glabra*[44,45], *Tinospora cordifolia*[72], *Picrorhiza kurroa*[23], *Alstonia scholaris*[73], *Cassia fistula*[74], *Sausserea lappa*[75], *Tylophora indica*[66], *Carum copticum*[76].

Many plants have been claimed to be useful in diabetes mellitus But the hypoglycaemic activity of several such plants is comparable to that of placebo. But as complementary medicines, several medicinal plants need to be investigated further because experimental and clinical studies have shown encouraging results - these medicinal plants are *Coccinea indica*[77], *Trigonella foenum -graceum*[78], *Azadirachta indica*[77], *Aegle marmelos*[78], *Enicostemma littorale*[78], etc. Actions other than hypoglycaemic activity need to be studied for viz. inhibition of protein glycosylation, reduction in microangiopathy, etc. Even there is enough scope to study the inhibitors of aldose reductase, insulinase, etc.

The interface of medicinal plant molecular pharmacology and modern molecular neuropsychiatry offers excellent opportunities for very productive research. In past, we could not adequately study the medicinal plants claimed to be useful for mind/brain disorders because the basic molecular mechanisms of these disorders were not known. Now with the information explosion in neurosciences, many active or other principles of medicinal plants can be studied for influence on diverse molecular phenomena 5-HT receptors-modulation, neuronal mRNA synthesis, Ca^{++} channels, NMDA receptors, Adenosine paracrine effects, effect on neuropeptides, etc. Some of the plants whose active principles need to be studied at the molecular levels are : *Centella asiatica*[80,81,82], *Withania somnifera*[83], *Nardostachys jatamansi*[84], *Evolvulus alsinoides*[85], *Celastrus paniculatus*[86] and *Mucuna pruriens*[87,88]

There are several plants mentioned as useful to women for diseases of the reproductive system - amenorrhoea, menorrhagia, leucorrhoea, diminished lacta-

tion, etc. With the current advances in the understanding of the neuroendocrine control of the menstrual cycle and the related disorders, there is a possibility to investigate these plants in a systematic way. Sizeable work has been done already e.g. *Adhatoda vasica* studies at R.R.L., Jammu[89]. But the developmental chain for follow-up need to be actively cultivated. Some of the plants which merit in depth studies, based on available data, are *Saraca indica*[90], *Mucuna pruriens*[illegible], *Hibiscus rosa-sinensis*[91], *Asparagus racemosus*[92], *Aloe indica*[93], *Symplocos racemosa*[94], *Ficus bengalensis*[95], *Ficus religiosa*[46], *Mimosa pudica*[96], *Lycium chinensis*[illegible], *Rheum* spp.[98], etc. The respective references for menorrhagia, ovulation induction, leukorrhoea, have to be looked up in the cited references of this review.

Viral infections, particularly those by the retroviruses and slow viruses constitute a major challenge to the scientists involved with research in chemotherapeutic or immune control of such viruses. Chemotherapy based on purine or pyrimidine analogues have limitations. More and more attention is being paid to cytokines - interferons, interleukins, macrophage activating factors, tumour necrosis factors, etc. But these substances *per se* have short half-lives and side effects. It is desirable to look for plants which influence these cytokines and hence the viral expression by the host cells. Many plants have been studied *in vitro* for antiviral activity but relatively less attention has been paid to the cytokines-induced modulation of viruses and the effects of medicinal plants. Some of the plants which merit such studies, based on the oral history or experimental/clinical data, are *Glycyrrhiza glabra*[45], *Picrorhiza kurroa*[illegible], *Berberis aristata*[49], etc. The work has to be carried out with isolated, pure principles of the plants in diverse *in vitro* and *in vivo* models, assaying cytokines.

Aging and ancillary disorders have engaged the interest of many scientists all over the world. Ayurveda and Chinese medicine have elaborate procedures described for rejuvenation. Some of the plants used for such studies need to be paid attention by gerontology research workers, both in animals and in man. There are chances that interesting neuroendocrine modulators may emerge from such an endeavour. The medicinal plants of interest are - *Phyllanthus emblica*[79], *Centella asiatica*[80], *Semicarpus anacardium*[100], *Glycyrrhiza glabra*[45], *Allium sativum*[99], *Boerhaavia diffusa*[47], *Asparagus adscendens*[101], *Panax ginseng*[102], etc.

Concluding Remarks

In a short review, it is difficult to be comprehensive and complete. Hence, I have chosen to be provocative to suggest fields for research so that the vast potential of medicinal plants, in therapy, can be explored. On a personal note, there is a four generation traditional knowledge base of Ayurveda in my family. And my work and research interest in plants is now for more than three decades. Young investigators, hopefully, will benefit from the review.

Our Prime Minister Sri P.V.Narasimha Rao said, "India has a rich tradition in Apara..... Vidya Ayurveda is one of these. It has contributed significantly to the health of the millions in our sub-continent and outside". Our scientific community needs to pay greater attention to the therapeutic legacy of Ayurveda and develop it further for the benefit of human kind[103].

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Drug Bioavailability Enhancement — A New Concept

Usha Zutshi and K.L.Bedi

Regional Research Laboratory
Canal Road, Jammu

SINCE the turn of century, product development in the pharmaceutical industry has evolved from cure-all herb teas to stable pure formulations containing known amounts of chemicals that have been defined as drugs. More recently we have come to the realization, sometimes surprising, that per cent chemical strength is not the only criterion for clinical efficacy. It became obvious that a dosage form must contain not only the correct amount of the labelled drugs but also release drug upon administration to the patient. Clinical effectiveness and bioavailability were thus added to the criteria for effective drug product development. A drug therefore, should not only be safe but beneficial as well and its therapeutic claims must be based upon sound clinical evidence.

What is Bioavailability?

The most important property of a dosage form is its ability to deliver the active ingredient to its site of action in sufficient amount to elicit the desired pharmacological response. This property of the dosage form has been variously referred to as its physiological availability or bioavailability. Bioavailability may therefore, be defined more precisely as an absolute term used to indicate the rate and the total amount of the drug absorbed from the dosage form and which then reaches the systemic circulation. According to this definition, the absorption of an intravenously administered drug is rapid and complete. However, for reasons of convenience and the stability, most drugs are administered orally after first being

formulated into a dosage form (delivery system), usually tablets or capsules. Therefore, their rate and extent of absorption in an individual are usually not precisely known. Since it becomes now clear that the therapeutic effect of a drug is a function of concentration of the drug in a patients's blood or plasma, the importance of bioavailability is too obvious to emphasize.

However, a large body of scientific evidence gathered over the years [illegible] clearly established that bioavailability may (and does vary) for a number of [illegible]. Such variations have been identified as causative factors in certain [illegible] therapy. Such therapeutic failures were seldom attributed to the product [illegible] to some abnormality of the patient. It is now evident that many [illegible] individually or in a concert may be the cause of therapeutic failure.

The reason for variations in bioavailability can be [illegible] either patient-related or dosage form related. Among the patient [illegible] those on which the physician and/or the patient can exert some [illegible] include the time of the drug administration relative to meals [illegible] emptying time), the co-administration of other drugs which may affect [illegible] and the compliance of the patient with the instructions of the physician, pharmacist or the nurse. Patient related factors which normally cannot be controlled, but for which allowance or adjustment can often be made, include age, disease state, genetic characteristics and/or gastro-intestinal physiology. Dosage form related factors which can produce profound differences in bioavailability include formulation and manufacturing variable such as particle size, the chemical form and the solubility of that drug, the type and the quantity of excipient used and the compaction pressure. The multitude of dosage forms related factors have consequently given rise to number of other characters strongly linked with the bioavailability.

Equivalence and Equivalent

Another term known as bioequivalence is equally important to understand, more particularly how the bioavailability and bioequivalence can affect the clinical outcome of the drug therapy. Equivalence is more a relative term and encompasses the comparison of one drug with another or a set established compendial standards. It can be defined and categorised in several forms.

Chemical equivalence: The drug products that contain the similar quantity of the same salt or derivative of the same active ingredient on the label and meet the compendial standards are stated to be chemically equivalent.

Bioequivalence: It implies that a drug in two or more similar dosage forms reaches the general circulation at the same relative rate and the same relative extent i.e. the plasma level profile of the drug obtained using the two dosage forms are reasonably superimposable.

Therapeutic equivalence: When one structurally different chemical can yield the same clinical results as another chemical, they are termed to be therapeutically equivalent.

Clinical equivalence: It occurs when the same drug from two or more dosage forms gives identical *in vivo* effects as measured by a pharmacological response or by control of a symptom or disease.

Obvious as it is, the drug products containing different salt or derivative of the same active ingredient, although not chemically equivalent and therefore, precluded by these definitions from bio- or therapeutically equivalent, may exhibit the same availability In other words, their administration produces comparable plasma concentration-time profile for the active ingredient. But each of these definitions provide atleast some basis for determining the equivalence or inequivalence of the drug forms. It is now widely accepted that bioequivalence studies, which compare the bioavailability of chemical equivalents, allow a meaningful assessment of the therapeutic equivalence of drug formulations.

In order to make bioequivalence more clear and comprehensive some suggestions have been proposed. One such proposal is replacing chemical equivalent with pharmaceutical equivalent The latter term is seemingly more acceptable because it comprehends a drug product (dosage form) whereas the term chemical equivalent tends to emphasize the chemical entity (drug) only contained in the dosage form. The two definitions, however, are otherwise apparently equivalent. Another significant proposal is the term pharmaceutical alternatives defined as drug products that contain the identical active therapeutic moiety but not in the same amount or dosage form, or not as the same salt or ester. For example, tetracycline hydrochloride capsules are pharmaceutical alternatives since the latter contain an amount of tetracycline base equivalent to that in 250 mg of the hydrochloride.

Such proposals can potentially obviate some of the problems associated with earlier definitions of bioequivalence in that they will permit non-chemically equivalent compounds exhibiting equivalent bioavailability to be considered bioequivalent and possibly therapeutically equivalent as well Previous definitions of bioequivalents have also been modified as follows. Bioequivalent drug products means pharmaceutical equivalents or pharmaceutical alternatives that when administered at the same molar dose of the active therapeutic moiety to the same dose individuals with the same dosage regimen (single or multiple dose) give equivalent bioavailabilities. Therapeutic equivalents mean pharmaceutical equivalents or pharmaceutical alternatives that provide essentially the same therapeutic effects when administered as labelled.

Absolute and Relative Bioavailability

Absolute bioavailability of a drug usually involves a comparison of areas under the plasma concentration-time curves (AUC) obtained following oral and intravenous administration (assuming instant and complete bioavailability) of the drug.

Relative (comparative) bioavailability is the comparison of AUC's obtained when like or unlike dosage forms of the same drug are administered. Thus, these studies may assess the relative bioavailability of capsules and suspensions, tablets and tablets, intramuscular injections and tablets, etc. However, relative bioavailability studies fail to provide estimates of absolute fraction of absorbed dose unless and until additional information such as urinary excretion data or blood level data from intravenous study is available. Therefore, a careful experiment design and proper statistical analysis of the data are prerequisites to reach valid conclusions regarding bioavailability. Relative bioavailability studies comparing drug absorption from chemically equivalent dosage forms are therefore, in essence, bioequivalence studies.

Incomplete Bioavailability

By and large, we are particularly concerned with the fraction of the oral dose that actually reaches the blood stream, because this amount is effective dose of the drug. In some cases particularly those involving drugs used as a single dose for acute purposes such as sedation, pain etc. we are also concerned with the rate of absorption of the drug. Many drugs are not completely available after oral administration, some have low permeability and are slowly absorbed even when given in solution; examples include cromolyn, neomycin and riboflavin. Since the residence of the drug at the absorption sites in gastrointestinal tract is limited by motility, there may be insufficient time for complete absorption. The availability of these compounds may be increased by administering them with food or with drugs that decrease motility, or by developing more permeable prodrugs.

Other drugs are so poorly water soluble that dissolution may be incomplete during the period of time available for absorption for example phenytoin, griseofulvin and isotretinoin. The availability of these drugs may be increased, in some cases dramatically, by dosage form changes, such as particle size reduction, or by means of soluble prodrug.

A surprisingly large number of drugs demonstrate incomplete availability because of chemical degradation in the stomach e.g. penicillin G, preabsorptive metabolism by enzymes in the proximal small intestine (aspirin) or bacteria in the distal small intestine and colon (digoxin), or presystemic metabolism in the gut wall (Isoproterenol) or liver (propranolol) during absorption. A drug subject to presystemic metabolism may be completely absorbed and incompletely available because part of the dose will be metabolised to other products during the drug's passage from the gut lumen to the systemic circulation. The availability of drugs subject to acid hydrolysis in the stomach may be improved by the use of enteric coated dosage forms.

History

The equivalence of therapeutic agents is a consideration that has been present in some form throughout ages. References expressing concern about drug's quality date back atleast as far as the fourth century BC. Theophrastus[1] noted that the quality of the medicines was related to the geographic origin of the plant source, the variety, the age, the portion of the plant used, and the method of harvesting, preservation and storage.

The earliest modern-day individual concerned with bioavailability concepts may have been Dr.W.E.Upjohn who, in 1885, patented the "Friabl" pill because of his desire to manufacture "pills" that would break down and be absorbed in the gastrointestinal tract in a reliable fashion[2]. Hence in 1902, suggested that a compressed tablet could contain the proper amount of ingredient and yet fall to produce the expected effect[3].

Passage of the Food, Drugs and Cosmetics Act of 1938 (USA) coincided with the beginning of a period of intense activity in pharmaceutical industry which saw the development of a number of new and important drugs. During 1940s, the time when Indian Drugs Act, 1940 was passed, the primary goal in the formulation of Drug products was to ensure that the dosage form contained the proper amount of active ingredient The official compendia therefore, were concerned with the establishment of standards for purity, potency and content

Oser *et al*[4]., in 1945 made one of the earliest suggestions that the official standards may not be adequate in assessing the performance of the drug products. The suggestion was made in a report concerning the absorption of water soluble vitamins from tablets. The authors reported that the physiologic availability of these vitamins varied considerably among the tablet they examined. However, this useful observation was almost lost for the next few years because of the lack of more such reports. Nevertheless, need for some objective measure of the release of drug from its dosage form was felt inevitable. Accordingly, in 1948, a laboratory procedure for determining the disintegration time of a tablet was adopted as an official test by the British Pharmacopoeia[5] followed by USP and National-Formulary[6] in 1950.

The disintegration test proved to be a valuable tool but provided no information concerning the rate of dissolution of drug from its dosage form though, as early as 1948, the importance[7] of this parameter in the control of biologic availability was recognised.

By late 1950s the limitations[8] of disintegration test came to be projected and in the early 1960s the significance of dissolution tests and their relationships between *in vitro* and *in vivo* results began to appear[9]. The utility of the dissolution tests was reviewed[10] by Morrison and Campbell in 1965 wherein the authors concluded that tablet disintegration tests had limited usefulness in measuring bioavailability and must eventually be replaced by more critical tests. Levy, in 1961, had also, in the mean time concluded[11] that while the tablet disintegration

test may be retained as an industrial control procedure but it has no value as an index of biovailability from dosage forms.

It was during 1960s that the bioavailability awareness also took its greatest leap forward. This awareness was provided by the emergence of a new science of "Biopharmaceutics". Dr. Gerhard Levy in 1960 used this term to describe the title of a new course at School of Pharmacy, University of California, Dr.J.G.Wagner in a comprehensive review[12] on drug absorption in 1961 defined Biopharmaceutics as the study of relationship of the physical and chemical property of the drug and its dosage forms to the biological effects observed on administration of the drug in its various dosage forms or in short the study of influence of formulation on the therapeutic activity of drug product.

Levy in 1960 cited[13] several examples of studies illustrating that the generic equivalency of drug product does not guarantee their therapeutic equivalency regardless of their manufacturer. A therapeutic failure resulting from a change in the brand of prednisone was reported[14]. A group of Australian physicians in 1970 also observed 15 number of patients exhibiting signs of phenytoin overdose which was later attributed to the use of different excipient in the capsule, a change made by the manufacturer resulting in greater bioavailability and toxic blood levels of the drug. The information amply illustrates the variable clinical response to the same dosage form of the same drug supplied by two or more different manufacturers.

The development of the discipline of Biopharmaceutics, coupled with the passage of Kefauver-Harris Amendments (Drug Amendments Act) in 1962 required the manufacturers to submit evidence of proof of efficacy and safety prior to marketing a new drug. This shifted the emphasis from a concern that the dosage form contained the identical active ingredients to greater regard for product formulation and the details of the manufacturing process. This development in USA has prompted many developing countries including India to make suitable amendments in their respective drug regulatory procedures. In India the inclusion of schedule Y in the Drugs and Cosmetics Act, 1940 is a step forward in this direction to achieve similar goals.

Bioavailability Enhancement

Conventional Approach

Manufacturers have tried to improve the bioavailability of the drugs by manipulating physicochemical characteristics of the drug by one or more of the following practices:

- micronisation of the active drug i.e. controlling the particle size
- deaggregation of the micronised particles by the use of protective colloids
- polymorphic/crystal size selection

- solubilisation of the active drug by
  (a) chemical derivatization
  (b) use of inclusion compounds such as with beta-cyclodextrine
  (c) use of co-solvents and surfactant system
  (d) complexation
  (e) by solid phase manipulation
- targetting and/or sustained release of the drug by:
  (a) film coating
  (b) using polymorphic matrices for sustained leaching
- prodrug approach with a view to increase absorption (both extent as well as rate), prevent first pass effect and target the drug on to the tissue or organ which needs, it most and possess requisite enzyme system to bioreverse or release the active drug. However, all these practices have their own problems and limitations.

New Concept

A new approach in the field of bioavailability was conceptualised while surveying the Ayurvedic literature[16-18] for certain herbals of medicinal significance. Frequent and consistent repetition of certain herbals individually/as a group was noticed in large number of prescriptions recommended for a variety of diseases. One of the group of herbals which is documented[19] very frequently as essential part of the prescriptions is "Trikatu" (trk) — the three acrids *viz.* long pepper (lp), black pepper (bp) and ginger (ging.) — in equal proportions by weight. It is hard to believe that this group of the herbals/individual components can be panacea for a variety of diseases for which these are prescribed.

Some modern Ayurvedic practitioners have tried to explain the scientific basis underlying the use of these herbals. According, to Dutt and King (1900) these herbals are added[20] to formulations often without reason and sometimes only for the sake of rhyme. Lakshmipathi (1940) reported[21] the usefulness of these acrids in the maintenance of balance of Kapha, Vatta and Pitta, the three humors of body according to Ayurveda. Bose (1928) was the only one who while describing the anti-asthmatic activity of Vasaka leaves mentioned[22] that addition of long pepper increases its efficacy.

The team at RRL, Jammu undertook to investigate the role of these plants, individually as well as in group (Trikatu) on kinetic behaviour of active drugs. One of the parameter adopted for the study was their influence on plasma drug levels or bioavailability of test drugs. Radioactive vasicine and spartein were taken initially as the test drugs

Two sets of experiments were organised for each drug. In one set of experiments vasicine alone was given orally and in another set vasicine was administered in combination with a suspension of finely powdered long pepper fruits

(250 mg/kg). Blood samples were monitored for radioactivity on addition of Bray's fluid, in a Beckman Liquid Scintillation System LS 3150-P using an external standard. Figure 1 displays vasicine concentration in the circulating blood monitored at different time intervals. It can be inferred from the data that co-administration of vasicine with long pepper alters the bioavailability of vasicine. Area under concentration versus time [AUC] is enhanced from 61.3 μg/ml to 204.4 μg/ml. It is also apparent from Figure 1 that serum levels of vasicine are maintained for longer period when vasicine is administered with long pepper. Quantatively there is 200% increase in bioavailability of vasicine under the influence of long pepper. The quantitative increase in bioavailability was computed by the procedure[23] adopted by Wagner (1975).

These results are the first scientific evidence describing use of these herbals or their active principle as bioavailability enhancer. It is also evident that these herbals were not included in the Ayurvedic formulations for the sake of rhyme but that they had definite role to play in increasing drug efficacy.

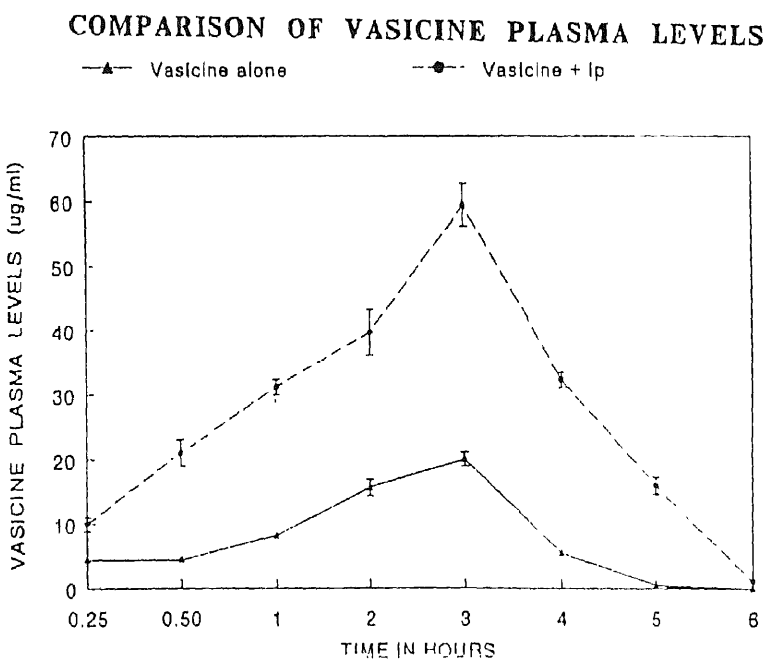

Fig. 1

In order to locate the component/chemical principle of Trikatu actually responsible for bioavailability enhancing activity, Trikatu as such, its individual components and the major chemical principle of *Piper* species were studied in detail. In this study ^{3}H vasicine was taken as test drug because of its easy and accurate

estimation method. *Pipper nigrum* Linn., *P.longum* Heum and *Zingiber officinalis* Rose were obtained from local market and authenticated. The major constituent (chemical) was isolated, purified to single entity (TLC,HPLC) and used in the study.

^{3}H vasicine was admixed with Trikatu, its individual components and RLJ-NE-210 in predetermined doses. The admixtures were administered [P.O.] to standardised groups of rats [n=6/group]. The mean plasma levels in different groups revealed that only component that could match Trikatu in toto was the pure compound RLJ- NE-210 (Fig.2). All other components of Trikatu exhibited an activity in fraction of the total.

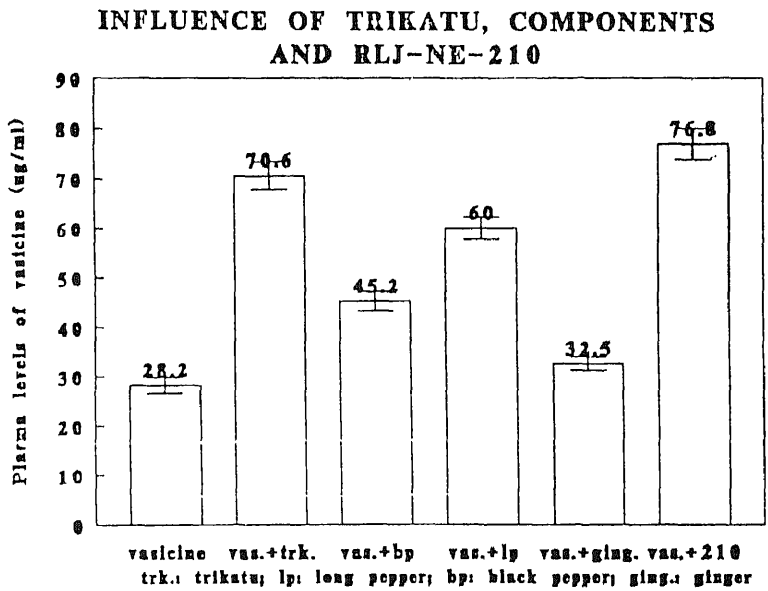

Fig. 2

Spartein: In this case only RLJ-NE–210 was used and administered in 30 mg/kg dose along with the test drug (Fig.3). Perusal of the concentration data obtained with and without co-administration of bioavailability enhancer (B.E.), revealed 100 % increase in AUC under the influence of this compound.

Development of New Concept of Bioavailability Enhancement

A large number of bioavailability and therapeutically different drugs which exhibited increase in their bioavailability in presence of RLJ-NE-210 were as under:

- rifampicin
- pyrazinamide
- isoniazid

COMPARISON OF SPARTEIN PLASMA LEVELS

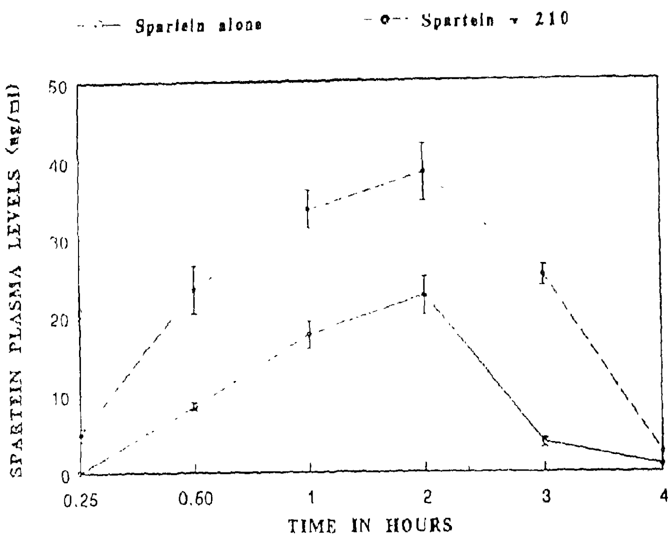

Fig. 3

- ethambutol
- dapsone
- tetracycline
- sulphadiazine
- 8-methoxypsoralen
- theophylline
- phenytoin
- astemizole
- loratidine
- chlorpheniramine
- ciprofloxacin
- propranolol

That the activity of the compound is not non-specific is confirmed by the fact that there are some other drugs whose bioavailability remains unaffected by RLJ-NE-210. The drugs are:

- tolbutamide
- chlorpropamide

- thioacetazone
- diazepam
- salmeterol
- salbutamol

Bioavailability enhancing property of RLJ-NE-210 was verified further and developed more elaborately by formulating it with anti-tuberculosis drugs.

Choice and Rationale of Comprehensive Evaluation of Bioavailability Enhancer

Preference for evaluation and development of Anti-tuberculosis [Anti-TB] formulations has been exercised mainly because of the following reasons:

(i) Tuberculosis is a disease that requires long term continuous therapy extending atleast upto 9 months. This incurs heavy cost and increases the risk of side effects.

(ii) Most of the Anti-TB drugs are hepatotoxic whereas isoniazid and ethambutol, in addition, can cause peripheral neuritis and opthalmic problems, respectively.

(iii) The most critical factor in Anti-TB Therapy is the plasma drug concentration to achieve maximum bactericidal & bacteriostatic action of the administered drug.

(iv) Except for streptomycin all Anti-TB drugs are administered orally. The bioavailability enhancer developed by RRL, Jammu has the advantage of oral administration and can thus be formulated conveniently with all these drugs.

Besides the above factors or compulsions for the preferential choice of the anti-TB drugs, the disease itself is dreaded as it is the leading cause of death among infectious diseases. Each year there are estimated 8 million new cases of TB and 2.9 million deaths from the disease. Around one-third of the world's population harbours Mycobacterium tuberculosis and is at risk of developing the disease. TB accounts for 6.7% of deaths in the developing world. Further, the steadily declining incidence of TB in the developed countries such as UK and USA has been reversed since 1985 with a large proportion of the cases infected with drug resistant mutants.

More seriously, the association between TB and HIV infection is now well recognised and accepted. According to WHO estimates, in 1992 around 4 million cases world wide have been infected with both HIV and TB, with about 95% in the developing world, TB in HIV cases is quite often due to highly compromised immune system and reactivation of earlier infections.

Clinical Evaluation of New Concept

After the acceptance of preclinical data by the Drugs Controller [India], due permissions have been granted stepwise for phased clinical trials of anti-TB formulations of RRL, Jammu.

Results of Phase-I and Phase-II Clinical Trials

As clear from Fig. 4 there is highly significant increase in Cmax of all the four anti-TB drugs evaluated in presence of bioavaliability enhancer. For example, the increase in Cmax of rifampicin, isoniazid, ethambutol and pyrazinamide is from 8.20 ± 0.46 to 17.65 ± 1.09, 3.56 ± 0.17 to 5.07 ± 0.90, 4.65 ± 0.83 to 7.50 ± 1.03 and 27.0 ± 2.0 to 40.0 ± 1.64 μg/ml, respectively. Similarly, ethambutol and isoniazid plasma Cmax in an ethambutol - isoniazid regimen are enhanced (Fig. 5)

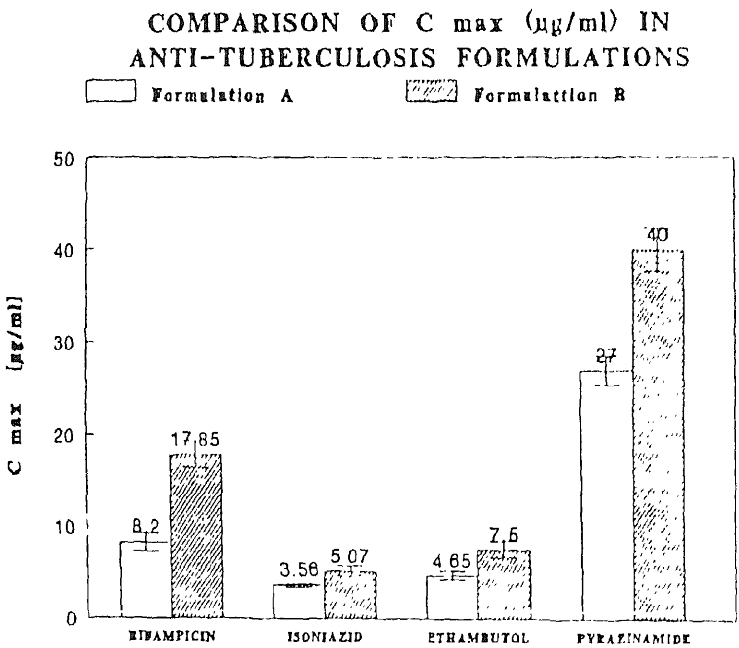

Fig. 4

by the bioavailability enhancer from 3.93 ± 0.49 to 6.78 ± 0.96 μg/ml and 4.09 ± 0.52 to 6.10 ± 0.38 μg/ml respectively. The reduction in absorption half-life of all the four drugs is equally significant (Fig. 6) suggesting an increased rate of absorption. Rifampicin, isoniazid, pyrazinamide and ethambutol exhibit reduction from 0.67± 0.008 to 0.40 ± 0.04, 0.64 ± 0.10 to 0.42 ± 0.04, 0.60 ± 0.03 to 0.37 ± 0.008 and 0.80 ± 0.04 to 0.41 ± 0.14, respectively. As the above two parameters are considered to be primary factors responsible for bioavailability, the highly significant increase in Cmax and decrease in absorption half-life of all the drugs are self- evident for bioavailability enhancing effect of RLJ-NE-210. Further, the elimination half-lives of all the drugs except isoniazid also exhibit highly significant increase (Fig. 7) and this coupled with the other two above said parameters obviously caused a highly significant increase in AUC (Fig. 8). This further

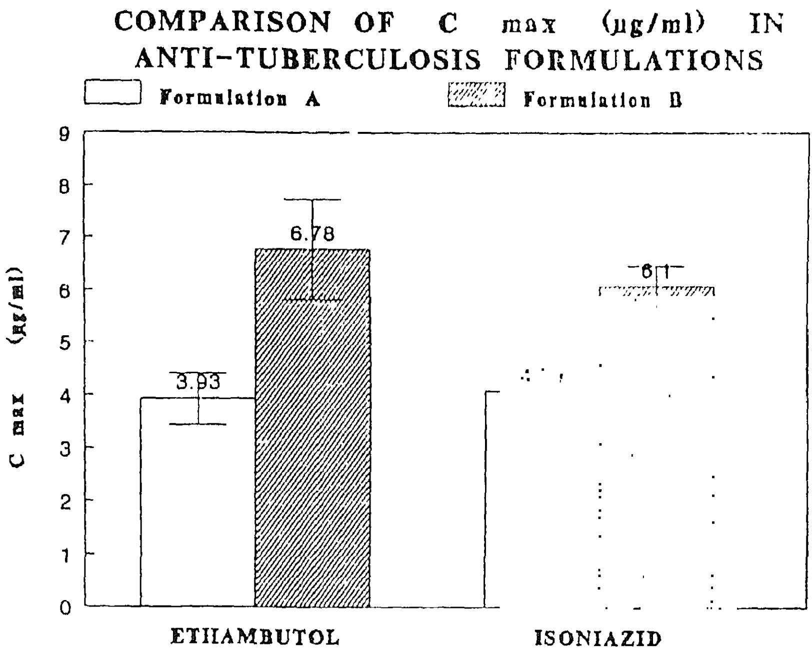
COMPARISON OF C max (µg/ml) IN ANTI-TUBERCULOSIS FORMULATIONS
Formulation A
Formulation B
C max (µg/ml)
3.93
6.78
ETHAMBUTOL
ISONIAZID

Fig. 5

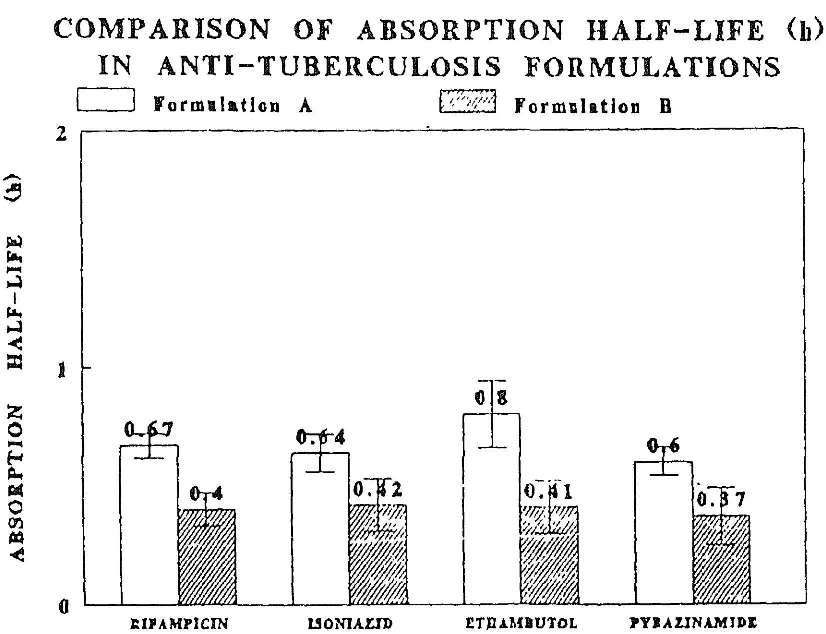
COMPARISON OF ABSORPTION HALF-LIFE (h) IN ANTI-TUBERCULOSIS FORMULATIONS
Formulation A
Formulation B
ABSORPTION HALF-LIFE (h)
0.67
0.4
0.64
0.42
0.8
0.41
0.6
0.37
RIFAMPICIN
ISONIAZID
ETHAMBUTOL
PYRAZINAMIDE

Fig. 6

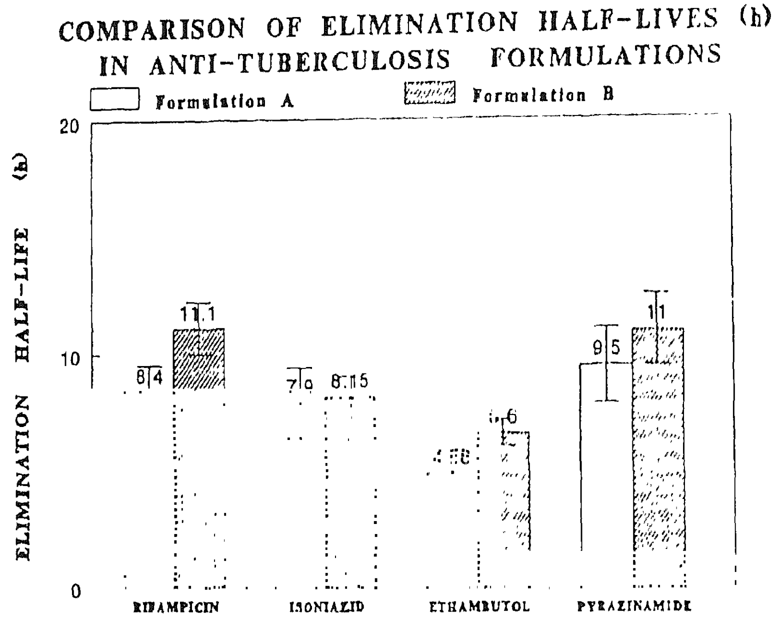
COMPARISON OF ELIMINATION HALF-LIVES (h)
IN ANTI-TUBERCULOSIS FORMULATIONS
Formulation A
Formulation B
ELIMINATION HALF-LIFE (h)
20
10
0
11.1
8.4
7.9
8.15
9.5
11
RIFAMPICIN
ISONIAZID
ETHAMBUTOL
PYRAZINAMIDE

Fig. 7

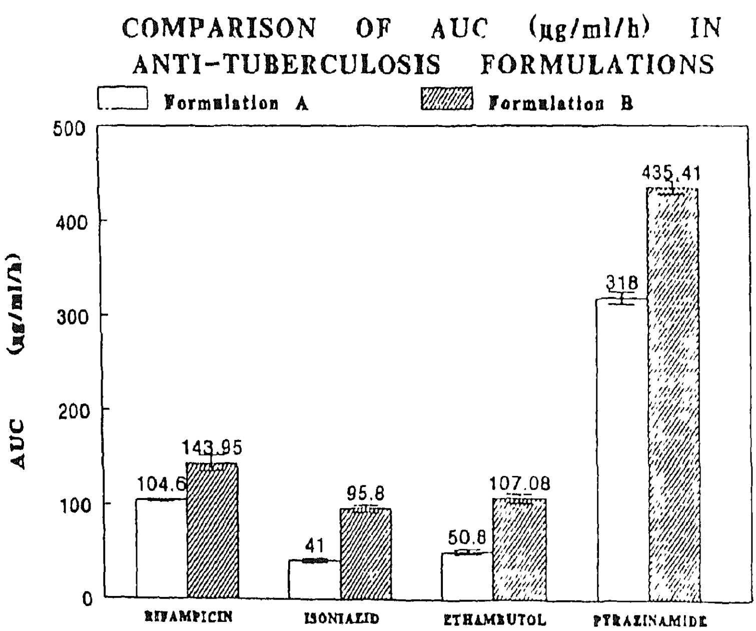
COMPARISON OF AUC (μg/ml/h) IN
ANTI-TUBERCULOSIS FORMULATIONS
Formulation A
Formulation B
AUC (μg/ml/h)
500
400
300
200
100
0
104.6
143.95
41
95.8
50.8
107.08
318
435.41
RIFAMPICIN
ISONIAZID
ETHAMBUTOL
PYRAZINAMIDE

Fig. 8

confirms enhancement in bioavailability. Besides, the most significant features of Phase-1 clinical trial has been the confirmation of reduction in the doses of active drugs proposed earlier in the preclinical dossiers. The degree and extent of reduction stands confirmed by the plasma drug levels (Fig.9) and the pharmacokinetic parameters. The figure exhibits comparative plasma drug levels of two different doses of rifampicin, as an example, in a formulation containing two or more other anti-TB drugs. It, therefore, confirms that 200 mg of rifampicin in presence of RLJ-NE210 has a bioequivalence to 450 mg of rifampicin without RLJ-NE-210. However, clinical equivalence of the two formulations was confirmed radiologically and other standard tests by the tuberculosis specialists during Phase-II clinical trials which finally clinches the claim made about the bioavailability enhancer - containing anti-TB formulations.

Phase-II

The aim of the Phase-II study was to validate the data generated during the preclinical and Phase-I clinical studies. The anti-TB drugs have been known and extremely researched for their efficacy against *Mycobacterium tuberculosis*. This was not the subject of the study. What was sought to be investigated was the clinical effect of combination of rifampicin with RLJ-NE-210 [formulation AT-3 designated as formulation "B" for the sake of convenience in this study]. This allowed

COMPARISON OF PLASMA LEVELS (μg/ml)
with formulation A & formulation B

--o-- Formulation A   --◆-- Formulation B

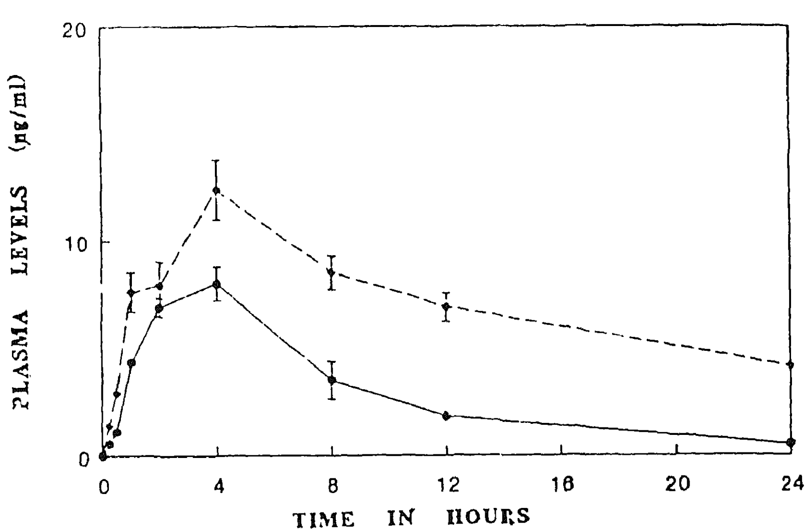

Fig. 9

the reduction of dose of rifampicin yet maintaining effective blood levels. This being so there was not much expectation in change in clinical profile.

However, the results obtained during Phase-II clinical trials confirmed that the reduced dose combination was as effective as the one in which RLJ-NE-210 was not used. The later formulation was designated as formulation "A". Also if anything, the clinical results were marginally better with formulation "B". Some of the indices monitored showed significantly positive early phase response. The weight gain was almost identical' the mean gain at 24 weeks being 5 kg for formulation "A" and 6 kg for formulation "B" (Fig. 10).

Disease score: Despite higher initial disease score for the group B, there was earlier change to favourable category and greater sense of well- being in this group. At the end of 24 weeks therapy this group had a higher percentage of "+" and "0" score patients when compared with group A based on radiological response.

The ESR showed significantly greater reduction (Fig. 11) in group B than in group A indicating favourable response to treatment.

The side effects observed during Phase-II clinical trials with formulations "A" and "B" were not unusual or new to the standard drugs [rifampicin, ethambutol and isoniazid] used in either of the formulations. Furthermore, the side effects were not found to be exaggerated in patients under treatment with formulation "B" as compared to those under treatment with formulation "A". The hepatotoxicity, normally associated with anti-TB drugs, was well controlled in patients under treatment with formulations "A" as well as "B" (Figs. 12 & 13).

Default Patients in Phase-II

There have been equal number of defaulters in both the groups i.e. A as well as B at different duration of therapy. The cause have been determined not to be related to any adverse effect of the therapy, but due to greater sense of well-being and clinical improvement felt by patients in group B at an earlier date. In group A default rate/number is same though this has happened at a much later stage of therapy as compared to group B. This again goes to prove that the earlier sense of well-being in Group-B might be the reason for an early default. The patients defaulted in both the groups at different timing after continuing regularly for 12 weeks. In group B defaulted patients had an initial score "++++" which was converted into "+" at the end of 12 weeks therapy. On the other hand, defaulted patients in group A had an initial score of "+++" which was converted into "+" in two patients and "++" in one patient at the end of 12 weeks therapy. This confirms that better and earlier clinical improvement was observed in patient treated with formulation B.

Highlights of Bioavailability Enhancing Effects of RLJ-NE-210

As already described in the text above the concept of traditional approach to bioavailability has been more of physico-chemical in nature. Different techniques used for the purpose would either help in better transport/solubilisation or involve

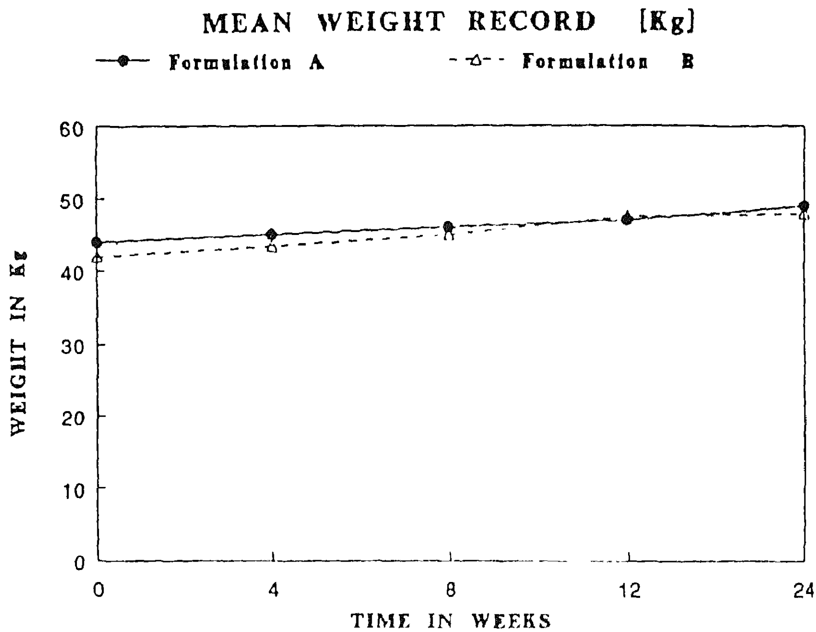
MEAN WEIGHT RECORD [Kg]
Formulation A
Formulation B
WEIGHT IN Kg
60
50
40
30
20
10
0
0
4
8
12
24
TIME IN WEEKS

Fig. 10

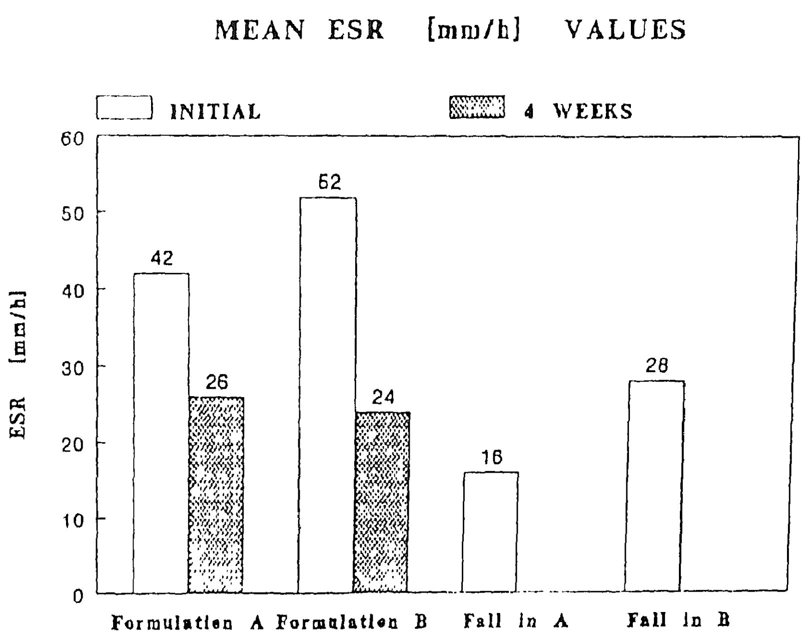
MEAN ESR [mm/h] VALUES
INITIAL
4 WEEKS
ESR [mm/h]
60
50
40
30
20
10
0
42
26
52
24
16
28
Formulation A
Formulation B
Fall in A
Fall in B

Fig. 11

MEAN OT VALUES IN PATIENTS UNDER PHASE - II CLINICAL TRIAL

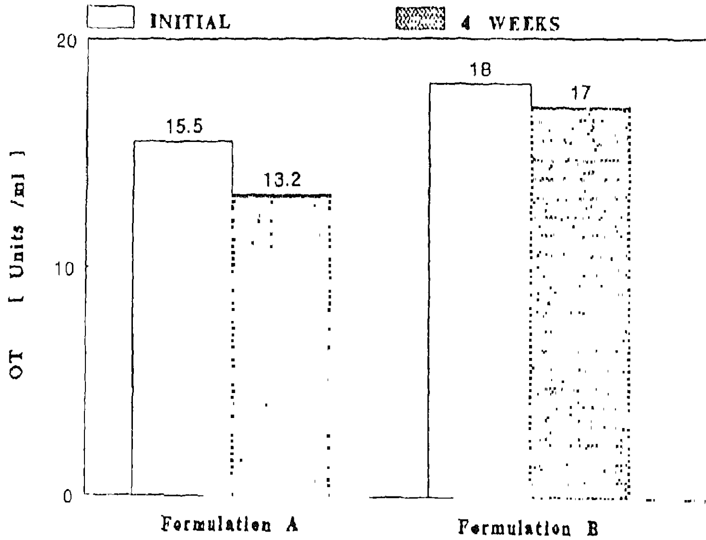

Fig. 12

MEAN PT VALUES IN PATIENTS UNDER PHASE- II CLINICAL TRIAL

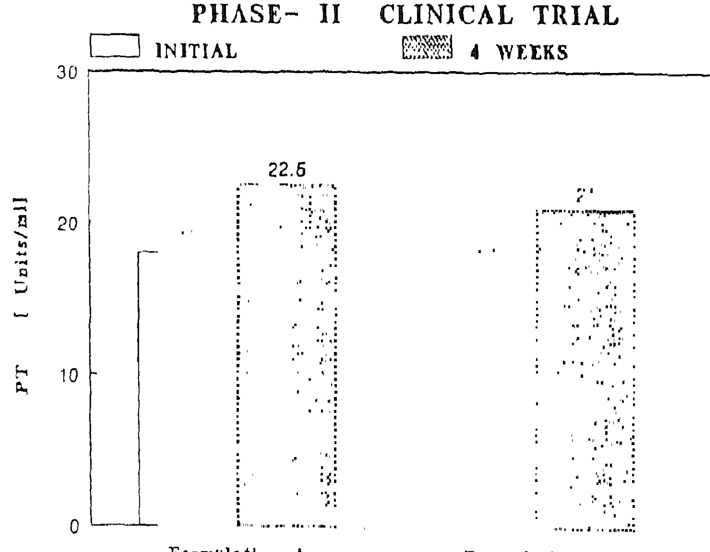

Fig. 13

chemical combination with the drug to facilitate absorption or carry the drug to the target tissue/organ. The new concept of bioavailability enhancer developed at RRL, Jammu is different and revolutionary in the following ways:

(i) This molecule can be termed perhaps a biological catalyst which reacts in a dose and time dependent, completely reversible manner with the bio-system both at transport as well as metabolism site.

(ii) The preference for its action on transport and/or metabolism levels depends upon structure of the drug with which it is formulated. This novel molecule causes a time dependent inhibition of metabolising enzymes when formulated with drugs suffering from faster elimination due to severe presystemic metabolism either in gut wall or liver.

(iii) Any change caused by this molecule, either at absorption or metabolic site is time dependent and completely reversible within few hours of terminating even its chronic administration.

(iv) The molecule is effective at very low doses irrespective of the dose or number of the drugs in a formulation.

(v) The presence of this BE in the formulation containing different drugs has been able to effect dose reduction of 40 to 60%.

(vi) The reduction in the dose and consequently the cost, especially of the expensive drugs such as rifampicin, ciprofloxacin is enough to drive home the economic advantage viewed at both national as well as global levels.

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Medicinal Plants – Priorities in Indian Medicines Diverse Studies and Implications

S.S. Handa*

Department of Pharmaceutical Sciences
Panjab University, Chandigarh - 160 014

IT has been estimated that from 250,000 to 750,000 species of higher (flowering plants) exist on earth; some of these have not yet been botanically described. Although there is no way to determine accurately how many of these species have been used in traditional medicine, a reasonable estimate would be about 10% or from 25,000 to 75,000 species[1]. However, perhaps only about 1% of these are acknowledged through scientific studies to have real therapeutic value when used in extract form by humans. Virtually all such plants have been discovered and put to wide spread use in traditional medical systems through information derived from their use in folk medicine, ethnomedicine or traditional medicine.

World population is nearing 5 billion today and with this rate of growth it is likely to touch 7.5 billion by the year 2020. Global estimates indicate that over 3/4th of the five billion world population cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plants. This fact is well compiled by W.H.O. in an inventory of medicinal plants list in over 20,000 species. As a part of strategy to reduce financial burden on developing countries which spend 40- 50% of their total health budget on drugs, W.H.O. currently encourages, recommends and promotes the inclusion of herbal drugs in national health care programmes because such drugs are easily available at a price within the reach of a common man and as such are

*Present address: Regional Research Laboratory, Jammu-180001 (J&K) India.

time tested and thus considered to be much safer than the modern synthetic drugs Resurgence of interest in herbal drugs in the western European countries is evident from the fact that two volumes of British Herbal Pharmacopoeia have been published and $ 33 million worth literature on herbal products was sold in the USA in 1990. The "green wave" in the utilization of medicinal plants resulted in higher consumption.

Medicinal Plants based Drug Industry

Medicinal plants based drug industry has four major segments, *viz.*, (i) plant drugs for Indian systems of medicine covering Ayurvedic, Unani and Sidha systems, (ii) over the counter, non-prescription items involving plant parts, extracts and galenicals, (iii) essential oils, and (iv) phytopharmaceuticals.

Medicinal Plants Based Drug Industries in Indian Systems of Medicine

There are at present 6,780 pharmacies in Indian Systems of Medicine of which 551 are on loan licence and the remaining 6,229 are on form D Besides, there are many small manufacturing units using medicinal plants and thousands of Vaidyas preparing their own drugs from various plants. Annual herbal drug production has been estimated at around Rs 800 crores and is expected to reach Rs. 4,000 crores by the year 2000. Various bottlenecks in the Indian herbal drug industry have been discussed elsewhere[2].

Plant Parts Extract and Galenicals

The direct utilization of plant material is a feature of traditional medicines not only in the developing world but also in Europe and the U.S.A., e.g., herbal formulations on health food shops. Preparations of decoctions, tinctures, galenicals and total extracts of plants also form a part of many pharmacopoeias of the world. The current trend of medicinal plants based drug industry is to procure standardized extracts of plants as raw material.

Essential Oils from Plants

The essential oil industry was traditionally a cottage industry in India. Since 1947, a number of industrial companies have been established for large scale production of essential oils, oleoresins and perfumes. The essential oils from plants being produced in India include ajowain oil, cedarwood oil, celery oil, citronella oil, davana oil, eucalyptus oil, geranium oil, lavender oil, lemongrass oil, *Mentha* oil, palmarosa oil, patchouli oil, rose oil, sandalwood oil, turpentine oil and vetiver oil. The manufacture of turpentine oil, and resin from pines is a sizable and well established industry in India having 10,000-25,000 tonnes annual production of the

oil. α- pinene and δ-3 carene are the two vital components produced from the oil. α- Ionone from lemongrass oil for perfumery and β-ionone for Vitamin A synthesis are produced in India. Before 1960, menthol was not produced in India but the introduction of Japanese mint, *Mentha arvensis* and subsequent improvements thereupon enabled India to produce over 500 tonnes of menthol and now tops the world market in export of natural menthol.

Annual world production of limonene is 50,000 tonnes and Brazil is the biggest producer in the world market. It is a by-product of citrus industry. Though turpentine oil and eucalyptus oil also yield limonene but the best economically cheap raw material is the discarded orange and lemon peel which is being used by Brazilian phytochemical industry. India has not yet tapped this source for limonene production.

Phytopharmaceuticals

Before 1947, the production of plant based modern drugs in India was confined to quinine from *Cinchona* in three state owned factories and the very first phytochemical industry for quinine was established by the then British Government at Mungpoo in Darjeeling. During the past three and half decades' bulk production of plant-based modern drugs has become an important segment of Indian pharmaceutical industry. Some of the phytopharmaceuticals which are produced in India at present include morphine, codeine, papaverine, thebaine, emetine, quinine, quinidine, digoxin, caffeine, hyoscine, hyoscyamine, xanthotoxin, psoralen, colchicine, rutin, berberine, vinblastine, vincristine, nicotine, strychnine, brucine, ergot alkaloids, senna glycosides, pyrethroids and podophyllotoxin resin.

Phytopharmaceuticals for which technology has been developed for undertaking large scale production include L-dopa from *Mucuna* beans, ajmaline and ajmalicine from *Rauvolfia serpentina* and *Catharanthus* roots, respectively, and 18 β-acetyl glycyrrhetic acid from *Glycyrrhiza glabra.*

Indian Institute of Chemical Technology (I.I.C.T.), Hyderabad has developed methods for etoposide and tenoposide production and CIPLA is now producing it on commercial basis. At present 100 mg of etoposide is sold at Rs.400/- per vial. National Chemical Laboratory, Pune, developed the method of vincristine (VCR) and vinblastine (VLB) production. CIPLA have further improved the process and now they are the third largest manufacturer of VCR and VLB in the world.

Medicinal plants based drug industry is progressing very fast in India, but it is beset with a number of problems. Most alarming problem the industry has started facing and will face in future is the dwindling supply of plant material from natural resources. A national policy on medicinal plants with a view to preserve endangered species and promoting cultivation of plants which are being extensively used by industry will help in solving the major problem of the industry. Special attention is required on medicinal plants on which significant research leads have been obtained

Table 1: Medicinal Plants on which Significant Research Leads have been Obtained

Commphora mukul	Antihypercholesterlaemic
Boswellia serrata	Antiarthiritic
Picrorrhiza kurroa	Antihepatotoxic
Phyllanthus amarus	Antihepatotoxic
Centella asiatica	Brain tonic
Curcuma longa	Antiinflammatory
Andrographis paniculata	Antihepatotoxic
Withania somnifera	Adaptogen
Coleus forskholi	Cardiotonic
Acorus calamus	Tranquillizer
Sidu rhombifolia	Anabolic
Albizzia lebeck	Immunomodulator
Valeriana wallichii	Tranquillizer

Table 2: Medicinal Plants being Imported

Glycyrrhiza glabra	Mullathi
Pimpinella anisum	Anise fruit
Thymus vulgaris	Hasha
Operculina turpenthum	Turbud
Cuscuta epithymum	Aftimum vilaiyti
Smilax ornata	Ushba
S. China	Chobchini
Lavandula stoechas	Ustukhudus

(Table 1), medicinal plants which are being imported (Table 2), medicinal plants having export potential and the threatened medicinal plants (Table 3).

Trade in medicinal plants is largely unorganised and uncertain, both in demand and price structure. There is a need to have Marketing & Development Board for Medicinal and Aromatic Plants and Phytopharmaceuticals. Such a Board could interact with the growers and user industry to bring stability in their production, demand, price, quality and also to help in fostering international trade.

India's Strength in Medicinal Plant Wealth

If we dwell for a moment on our hoary past, Rigveda, ne of the oldest repositories of human knowledge written between 4500-1500 BC mentions the use of 67 plants for therapeutic use and Yajurveda enlists 81 plants whereas Atharveda written

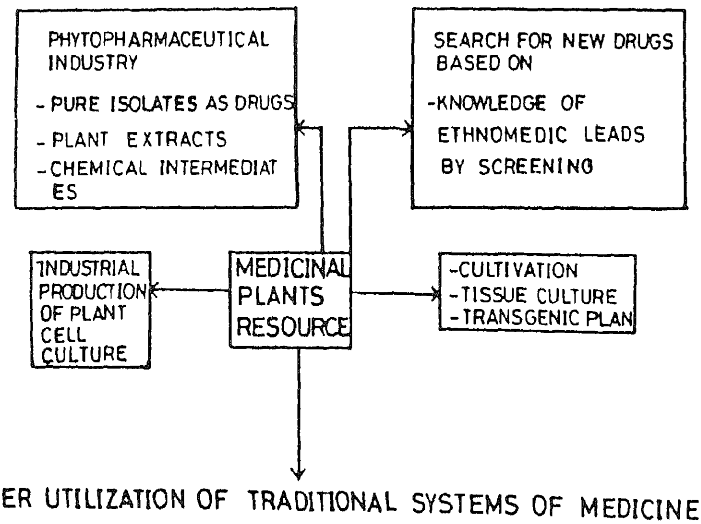
Table 3: Threatened (Endangered) Medicinal Plants

Name	Present status
Aconitum deinorrhizum	Almost extinct
A. heterophyllum	Greatly threatened
Angelica glauca	Threatened
Arnebia benthemii	Threatened
Artemisia brevifolia	Likely to be threatened
A. maritima	Likely to be threatened
Atropa accuminata	Threatened
Berberis aristata	Threatened
Bunium persicum	Greatly threatned
Colchicum luteum	Threatened
Corydalis govaniana	Likely to be threatened
Dactylorhiza hatagirea (orchis)	Threatened
Dioscorea deltoidea	Threatened
Ephedra gerardiana	Likely to be threatened
Ferula jaeschkeana	Threatened
Gentiana kurroa	Threatened
Hedychium spicatum	Likely to be threatened
Jurinea dolomiaea (Dhoop)	Likely to be threatened
Nardostachys jatamansi	Threatened
Orchis latifolia	Threatened
Picrorrhiza kurroa	Likely to be threatened
Podophyllum emodi	Threatened
Rheum emodi	Threatened
Swertia chirata	Threatened
Valerian wallichii	Likely to be threatened
Zanthoxylum alatum	Likely to be threatened

during 1200 BC describes 290 plants of medicinal values. "Chakra Samhita" (~900 BC) describes 341 medicinal plants and the next land mark in Ayurveda "Sushruta Samhita" (~ 600 BC) mentions 395 medicinal plants[3]. India unquestionably occupies the top position in the use of herbal drugs. It is one of the foremost countries exporting plant drugs and their derivatives, and excels in home-consumption too and this is not all surprising because of the following:

- great biodiversity and abundance of flora
- variety of geographical climatic conditions most exotic medicinal plants can be grown here

DRUGS FROM PLANTS : AN INTEGRATED APPROACH TO DEVELOPMENT



- QUALITY CONTROL STANDARDS
- MODERNIZATION OF METHODS OF PRODUCTS
- NEW DOSAGE FORMS & PHARMACOKINETICS
- SAFETY OF DRUGS USED IN CHRONIC DISEASES
- INTEGRATION WITH MODERN MEDICINE

Fig.A. MEDICINAL PLANTS RESOURCE

- Indian systems of medicine dwell heavily on medicinal plants
- long tradition of phytochemical research and scientific cultivation of medicinal plants
- well developed pharmaceutical industry
- rapidly growing phytochemical and herbal drug industry

India is thus in a vantage position to exploit this source both for meeting the domestic demand for drugs as also for export.

Medicinal plants continue to be an important resource material for therapeutic agents both in developed and developing countries[4-7]. The Figure A shows the medicinal plants resource and better utilization of traditional systems of medicine.

Priorities in Research on Medicinal Plants & Diverse Studies

It is desirable to have a "need based" approach to research on medicinal plants including screening of plants for biological activity. This was the objective of a W.H.O. Regional Group which met in 1980. Research efforts could thus be directed for a number of diseases for which suitable drugs are not available in the modern system of medicine and where herbal drugs have a possibility of offering new drugs, some such conditions include the following where new drugs are urgently needed and the medicinal plants have already provided some leads:

Antiprotozoal Drugs from Plants

Tropical diseases including malaria where tissue schizontocidal drugs are needed and drugs for multi-drug resistant blood schizonts are required. It has been estimated that one third of the world's population may be exposed to the risk of malarial infection[8,9]. Most hard hit areas include Africa, India, China and East Asia. Amoebiasis caused by *Entamoeba histolytica* is the major cause of dysentery in the developing world where an estimated 42 million cases occur annually and untreated disease may progress to hepatic amoebiasis and other complications which are responsible for 75,000 deaths each year[10]. Filaria and leishmania are two other protozoal diseases affecting our population. Many plants like *Peganum harmala, Celastrus paniculata, Artemisia annua, Berberis aristata, Tilicora triandra* have been reported [11,12] to have activities against various species of protozoa and a variety of such plants have been well illustrated in several recent reviews[13-16]. For amoebicidal drugs plants like *Holarrhena antidysenterica*, *Berberis aristata*[17], *Allium sativum*[18] and *Terminalia belerica* [19] need serious attention. Worldwide it has been estimated that 20 million people are infected with *Leishmania* species and that 400,000 new cases occur each year[20]. The plants which have given leads

include *Plumbago zeylanica*[21], *Diospyros montana*[22], *Ricinus communis* and *Phytolacca* species[23].

Antiulcer Drugs from Plants

Important plants for the treatment of gastrointestinal ulcers that have promising prospectus as useful drugs include *Prosopis glandulosa, Calendula* species, *Artocarpus integra, Musa ferrea* (pulp) and deglycyrrhizinated *Glycyrrhiza glabra* and many others which have been reviewed by Lewis & Hanson[24].

Antirheumatic Plants

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases of mankind and affects a large population of the world, no substantial progress has been so far achieved for permanent cure. Our recent review reveals that plant species of ninety six genera belonging to fifty six families have exhibited antiinflammatory activity[25] Most significant plants include *Aesculus hippocastanum, Azadirachta indica, Boswellia serrata, Commiphora mukul, Curcuma longa, Ochrocarpus longifolia, Pluchea lanceolata* and *Vitex negundo*[26-28].

Antidiabetic Plants

15.2 Million people are suffering from diabetes in our country and the number is increasing every year. About 148 plants of 50 families reported to have hypoglycaemic activity have been reviewed by us[29]. The most important hypoglycaemic plants which need serious clinical trials include *Pterocarpus marsupium, Momordica charantia, Trigonella foenum-graecum, Salacia prinoides, Gymnema sylvestris* and *Cyamompis tetragonolobus*.

Antiasthmatic Plants

In the lack of any permanent cure in the modern system of medicine for bronchial asthma, plants like *Albizzia lebeck* which have immunomodulatory action need serious attention. Other poly-herbal Ayurvedic formulations like Yastiadivati and Shereeshadi Kashaya may prove to be efficaceous[30]

Antiviral Plants

Experimental work on a number of plants having antiviral activity has been done[31-34]. A lyophilized infusion from flowers of *Sambucus nigra*, aerial parts of *Hypericum perforatum* and roots of *Saponaria officinalis* exhibited antiviral effect inhibiting reproduction of different strains of influenza virus types A & B, both *in vitro* and *in vivo* and herpes simplex virus type 1 *in vitro*[35]. These plants need to be carefully examined.

Plant adaptogens:The notion of "resistance" to disease and the idea that such resistance can be modified by life experience and by emotional states, forms one of the basic tenets of Ayurveda thus avoiding the cartesian dichotomization of mind and body[36]. Concept of Rasaayana and Rasaayan plants have been reviewed by the

author[37,38]. Plant of interest in this area include *Withania somnifera, Ocimum sanctum, Picrorhiza kurroa, Asparagus racemosus, Pueraria tuberosa, Sida cordifolia, Desmodium gangeticum, Boerhaavia diffusa* and *Cissampelos pareira*. We need to develop suitable models for testing such vital plants of Ayurveda.

Hepatoprotective Plants

A global estimate indicates that there are about 18,000 deaths every year because of the liver cirrhosis mainly caused by hepatitis. Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2,50,000 new cases each year. Although viruses are the main cause of liver diseases, the liver lesions arising from xenobiotics, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon. Modern drugs have very little to offer for alleviation of hepatic ailments, whereas, most important representatives of phytoconstituents used for liver diseases, chiefly on regional basis, include drugs like silymarin (*Silybum marianum*), catechin (*Anacardium occidentalis* and others) in Europe, glycyrrhizin (Glycyrrhiza glabra) in Japan and schizandrins (*Schizandra chinensis*) in China[39]. In India, we have over 40 polyherbal commercial formulations reputed to have hepatoprotective action. A review published on the subject from our laboratory indicates that 160 phytoconstituents from 101 plants belonging to 52 families have antihepatotoxic activity[40,41]. Our laboratory has worked extensively on *Andrographis paniculata*[42] and hepatoprotective activity has been established due to the presence of andrographolide. Kutkoside from *Picrorhiza kurroa* is a potential hepatoprotectant reported by CDRI[43-47]. *Phyllanthus amarus* is another most important plant selected for clinical trials[48]. Our future work in this area is bound to give fruitful dividends. Antihepatotoxic activity of *Boerhavia diffusa* and *B. repanda* has been also reporoted from our laboratory[49]. Kolaviron, a mixture of *Garcinia kola* (Guttiferae) biflavonoids at a dose of 100 mg/kg i.p. prevented thioacetamide induced hepatotoxicity[50]. *Withania fruitescens* (Solanaceae) leaves exhibited protective and curative action against carbontetrachloride induced liver toxicity[51]. Plant kingdom appears to be a fruitful ground for the discovery of effective hepatoprotective drugs which we currently lack in the modern system of medicine.

Fertility Control by Plants

The successful introdution of three plants into modern therapeutics and family planning in the last forty years indicates that there are other discoveries waiting to be made[52]. Many indigenous plant preparations are prescribed by Ayurvedic physicians in India from time immemorial for the prevention of conception. Clinical evaluation of such plant preparation is called for on priority basis.

Anti-cancer Drugs from Plants

Cancer is an insidious disease affecting mankind in every country. Work on periwinkle plant, *Catharanthus roseus* (L >) G. Don was independently taken up in two different laboratories for its alleged hypoglycaemic activity as per Jamaica

folklore. Though none of the groups could substantiate hypoglycaemic activity, the Canadian group of Nobel Beer and Cutts succeeded in isolating vinblastine while Eli Lilly group headed by Svoboda could isolate vinblastine and vincristine along with two other active dimeric alkaloids. These alkaloids are present in exceedingly low concentrations in a complex mixture of 50 alkaloids[53]. Vinblastine was introduced (Velban, Eli Lilly) in 1961 and vincristine (Oncovin, Eli Lilly) in 1963 as anticancer drugs. CIPLA has improved upon the process of isolating vinblastine and vincristine from *Catharanthus roseus* as developed by NCL, Pune. Today we are the third largest manufacturer of vinblastine and vincristine in the world and we are exporting these alkaloids to European countries and the demand is steadily increasing[54].

Screening of plant extracts for anticancer activity started in 1961 by National Cancer Institute of the U.S.A., and up to 1981 (20 years) about 1,14,045 plants had been screened, of which only 3.4% (representing about 3,400 different species) have been observed to be active in one or more bioassay systems. The promising phytoconstitutents which are likely candidates for drug development include indicine N-oxide (a pyrrolizidine alkaloid) from *Heliotropium indicum*, ellipticine (a monomeric indole alkaloid) from several *Ochrosia* species, homoharringtonine (a cephalotaxine alkaloid) from *Cephalotaxus* species, taxol[55] from *Taxus* species and camptothecine (quinoline alkaloid) from *Camptotheca acuminata* a Chinese tree)[56]. The author has been successful in discovering a number of anticancer agents from plants like *Ostodes paniculata*[57], *Feddiea fischeri*[58], *Soulamea soulameoides*[59], *Dirca occidentalis*[60], and *Passerina vulgaris*[61]. The anticancer principles of podophyllum are contained in the resin, podophyllum resin or podophyllin. American podophyllum yields 2-8% and Indian podophyllum about 6-12% of the resin. It is not only the higher amount of the resin present in Indian podophyllum (*P. emodi* var. *hexandrum*) but there are no peltatins present in contrast to American podophyllum (*P. peltatum*) which contains α- and β- peltatins. Thus, the Indian podophyllum has higher amount of podophyllotoxin. In certain cases Indian podophyllum has yielded 20% resin. The highest amount is in May when the plant produces flowers. Thus the Indian podophyllum, when collected at the proper season not only contains 2-1/2 times or even more of the resin compared to American podophyllum, but this resin has double the amount of podophyllotoxin which is the active principle used by pharmaceutical industry for structural modifications to produce anticancer drugs tenoposide and etoposide being marketed by Sandoz. Major problem with the cultivation of this plant is that the seeds take very long time to germinate. We need to concentrate on cultivation of this plant either by propagation or by reducing the period of seed germination. Use of Taxol from *Taxus brevifolia* or *Taxus baccata* is the latest addition of anticancer drug especially in ovarian cancer[62].

Plants for Urinary Stones

The very first mention of 'Pashanbhed' in Ayurvedic literature is in Charak Samhita, and is recommended for painful micturition and for breaking calculi. Sushrut Samhita mentions the drug under various synonyms - Pashanbhed for uric acid calculi and Ashnibhed for biliary calculi. Sushrut Samhita mentions decoction of Pashanbhed, Astimantaka, Satvari, Vrihati, Bhalluka, Varuna (*Crataeva nervula*), Kultha (*Dolichos biflorus*), kola and katak seeds for patients of Vataja Ashmari while Kusa, Ashmabhed, Patala, Trikanthaka, Sirisha, Punarnava (Boerhavia diffusa) and shilajit are for Pittaja Ashmari. About 4000 plants have been mentioned to be useful for dissolving stones in the urinary system in a review published seven years ago[63]. Cystone of Himalaya Drug Co. and Calcury of Charak Pharmaceuticals are already in the market. *Crataeva nervula* bark and *Tribulus terrestris* fruits have been put under clinical trials by Indian Council of Medical Research. *Cucumis trigonus* (curcurbitaceae) has been investigated for its diuretic activity[64].

Plants as Sedatives/Tranquillizers

Indian valerian (*Valeriana wallichii*) contains 2% valepotriates and is thus four times more potent than European valerian (*V. officinalis*) which contains 0.5% valepotriates. These are triesters of polyhydroxy cyclopentanopyran esterified with isovaleric, acetic and β-acetoxy valeric acid. Compared to known iridoids, valtrats (valepotriates) are neither glycosides nor lectones and are considered original or primary products present in the drug responsible for activity. Valtrates are used as tranquillizers and sedatives, and the action is comparable to meprobmate. Additional advantage is that these can be prescribed to alcoholic patients. Valerian also contains valernic acid having spasmolytic action. Valeranone is found in jatamansi (*Nardostachys jatamansi*) possessing sedative property. It will be worthwhile to produce valepotriates in India. A peruvian plant *Valeriana thalictroides* has been found to contain 14.5% total valepotriate content in the roots and thus yields the highest known concentration of valepotriates in plants to date[65]. The high yield of valepotriates in *V. thalictroides* roots cannot be directly used to overcome shortage of valepotraites for therapeutic use, as the roots have very small diameter (2 cm) and a length of about 5 cm. *V. edulis* ssp procera (HBK) F.G. Mey, although having a smaller valepotriate content (7%), is a much better source for valepotriates because of its higher drug yield. However, such high yielding plants could be used to establish high yielding tissue culture for the *in vitro* production of valepotriates. Relation between valepotriate content and differentiation level in various tissues from valerianaceae has been studied[66]. Isolation and evaluation of valepotriates from Indian valerian are well known[67].

Plant Laxatives

Constipation is a common problem of Western and European countries because of protein rich diet. In the U.S.A , 1000 patents of vegetable origin are there which

involves an annual trade of $ 500 million. On an average we export 15,000 tonnes of plantago, 7000 tonnes of senna and 1000 tonnes of rhubarb annually. Trade in plant laxatives increases by over 10% every year. India is the sole supplier of *Plantago ovata* seeds and husk (psyllium) in the international market. India's export of Psyllium seeds in 1980-81 were to the extent of 25,743 metric tonnes valued at Rs.85.93 million. Indian Institute of Management, Ahmedabad has published recently a survey report on the psyllium production and marketing in India[68].

Pitfalls in Plant-drug Research and Implications

Medicinal plant research is beset with a number of problems and the major stumbling blocks encountered in plant drug research are listed below:

(a) Medicinal plants being used by traditional practitioners in India not only bear different names in different geogrpahical areas and in different languages, but it is also not uncommon that different plants are known by the same name which add to the confusion and has resulted in controversial identity of many plants[69].

(b) Major causes of inconsistancy in plant drugs include ontogenetic, ecotypic, genotypic and chemotypic besides variations due to harvesting period, method of drying and storage conditions.

(c) Ayurvedic, unani practitioners invariably use polyherbal formulations, whereas, for obvious reasons, it is easier for the modern scientists to investigate single plant drug.

(d) Appropriate experimental models are not available for validating claims of some very important and useful plant drugs like "Rasaayana" enjoying esteemed repute in Ayurveda and termed in modern terminology as vitalizers, rejuvenators, adaptogens, immunomodulators etc.

(e) Major national laboratories resorted to more broad based screening of plants[70] by including, apart from plants which are well known and time tested by traditional systems of medicine, those which are not mentioned by these systems. Others undertook studies largely on individual initiatives. Inspite of all these efforts, however, the ultimate goal of producing inexpensive, safe and efficaceous drugs for most diseases encountered in our country remains to be achieved.

(f) The major bottleneck, however, is the lack of scientifically planned clinical trials on traditionally used medicinal plants. Though research work on medicinal plants has been carried out in Indian research establishments since long but not much has been achieved. The beneficial phase of a multidisciplinary integrated approach to the evaluation of plants initiated by the Composite Drug Research Scheme seems to have been almost lost in the pages of history. Recently, however, Indian Council of Medical Research has adopted new

strategy of disease oriented approach of carrying out clinical trials on medicinal plants selecting certain 'refractory diseases' i.e. those for which modern medicine has not been able to offer, so far, a satisfactory or a lasting remedy.

(g) The novelty and popular trend on the part of the phytochemists and pharmacologists to continue efforts to find out an "active principle" responsible for the pharmacological action and therapeutic efficacy of a plant, in many instances, has also led to frustrating results. Pharmacologists and phytochemists engaged in medicinal plant research may have to change their approach to wholistic plant material or with semi pure principles or even crude extracts. Gum guggul (*Commiphora mukul*) is a classic example.

(h) Except for Central Drug Research Institute, Lucknow, little facilities exist elsewhere in India for carrying out pre-clinical evaluation including toxicity tests on medicinal plants.

(i) Clinical evaluation of plant drugs is an area which is handicapped with lack of facilities and proper orientation of scientists of different disciplines. At present, for lack of such facilities even the leads obtained so far are being lost to follow-up.

Herbal Drug/Intellectual Property Rights (IPR)

Medicinal plant research has been and continues to be considered as a fruitful approach for the search of new drugs. The highly respected magazine *Science* calls attention in its editorial of February, 1990 to the potential of medicinal plant in the search of new drugs[71] and expresses concern about the consequences that loss of biodiversity might have in this context. Since most of the chemically unknown flora and associated medicinal lore exist in the developing and underdeveloped countries, especially those which still possess extensive tropical forests, the rapid loss of biodiversity does indeed become an issue for the future of medicinal plant research. The traditional role of developing countries in plant drug production has been as suppliers of raw material. Most of the pharmacological and chemical studies reported in several journals start by quoting the traditional use of a given plant species promising enough to stimulate further research. It is, therefore, reasonable to assume that the indigenous knowledge did play an important role in medicinal plant research and will continue to do so. Posey[72] calls for legislation to secure Intellectual Property Right for indigenous people.

Herbal Drug Trade

The extent of herbal drug usage is hard to quantify for traditional systems of medicine in India as by tradition many of the practitioner manufacture and formulate their own prescription, although there are now about ten well organised large scale herbal drug manufacturers whose individual annual production is of about $ 112 million (June 1988). Their total annual turnover is about $ 350 million[73]. In

U.K. there are about 5,500 herbal products involving 1,600 herbal ingredients. It is estimated that some 6,000-7,000 tons of herbs are extracted annually for the ingredients in U.K. medicine[74]. Like India, China is another country which is noted for its adherence to herbal medicines for a number of therapies like in the treatment of abdominal conditions (appendicitis, perforated ulcers, pancreatitis), arthritic disease and some neurological disease. There are about 248 herbal drugs used for a number of reasons e.g., analgesic sedative, diuretic, anti-tussive, antirehumatic and antiasthmatics[75].

Herbs and the literature about them are big business in America. In 1985 the sales of all forms of herbs exceeded $ 190 million. Unfortunately, most of the literature prepared with a single purpose in mind-to sell a product, since to do so most effectively, it is necessary to promote or advocate all of the good features of the herb while minimizing, or even omitting, any negative aspects, such writings are generally referred to as advocacy literature[76].

Current federal laws do not permit the sale of herbs as drugs, that is, with claims of efficacy appearing on their labels. If such claims are made, the efficacy must be proven to the satisfaction of the Federal Food & Drug Administration, a process as much as $ 100 million per drug. Since no one is willing to spend this amount of money on a product for which patent protection is not available, herbs are sold labelled only with the name of the product, primarily in 'health food' stores. Literature purporting to explain the uses of herbs is also available in such establishments. Because the laws and regulations applied to food naturally do not require any proof of efficacy prior to sale, some manufacturers attempt to market herbal products as nutrients or food supplements, by combining a variety of herbs with standard multivitamin mineral preparation and often make extravagant claim for the products based on their herbal contents.

Herbal Medicines – Medicinal Marvel or Money Spinning Malarkey

Ayurveda, with many centuries of experience and cultural support has strengths which should be used only through the experts and vaidyas in Ayurveda or by modern physicians, who have undergone practical training and experience in Ayurveda. But gross and rampant commercialization of Ayurvedic herbal remedies, by all and sundry, has to be criticized and even condemned. With the onslaught of media television, radio and press several herbal formulations are promoted, often indiscriminately. Consumer associations are very quick and rightly so, when unscientific claims are made by ethical modern drugs. Should they not take up issue with very tall claims by the so-called herbal drugs by commercial interests? There is an urgent need to create an ethical code for advertising and promotion of Ayurvedic drugs.

Sociopolitical, Economical & Ethical Issues in Medicinal Plants Research

Due to enormous difference of wealth generated by supplying crude medicinal plant material versus the purified isolated phytoconstituent, the developing countries remain the worst sufferers. It therefore, calls for legislation to secure Intellectual Property Right (IPR) for indigenous people. What are the ethics behind utilizing traditional knowledge without adequate compensation to the societies from which it originates.

Herbal Drug Development

One may propose a development procedure tailor made for herbal drugs. Traditional medicine usually consists of a mixture of herbal drugs.

In the first step the therapeutic efficacy of the traditional medicine has to be confirmed. Of course, the composition of the recipe has to be affirmed and authenticated by Pharmacognostic methods which means that we have got to be sure which plant, identified by botanical nomenclature, and which part of the plant to which percentage is used. Only in the country where the recipe has been used traditionally clinical trials can be performed. These trials have to be carried out as scientifically as possible. Well defined clinical parameters have to be evaluated in a population of patients with well defined diagnosis. In India such clinical trials on traditional medicines have been initiated by the Indian Council of Medical Research adopting disease oriented approach.

In the positive outcome of such studies we proceed to the second step. We have to select one, perhaps in some cases two or three plants as the active ingredients. This can be done by animal pharmacology or clinical pharmacology. At this stage we have to attempt standardization employing all possible means like finger printing, TLC, HPLC or other related techniques. The clinical trials are performed against a placebo. In each case we have to use as much well defined clinical parameters as possible in order to establish the efficacy of the drug. If the results are positive, we can proceed to the next step where we use purified extract being standardized for the reprocessed extract, we can establish mode of action and also carry out toxicological studies in animals which become mandatory since we now use much higher concentration of the drug than used in the original recipe. With this standardized extrtact we can perform placebo controlled clinical trials which still should be performed in the country of origin. Nevertheless, we make our first attempt to submit something like Investigational New Drug (IND) in order to be allowed to perform phase I or even phase II clinical studies outside the country of origin. If the results are positive we can proceed to internationalization of the product. There are some countries in the world where the health authorities have a positive attitude to herbal drugs. In these countries facilitated approval may be

possible. They can be fore runners for other countries where the developmental requirement would be the same as the synthetic compounds[77].

Standardization of Herbal Drugs

The single and most important factor which stands in the way of wider acceptance of traditional herbal medicines is the non-availability or inadequacy of standards for checking their quality by chemical or bioassay methods. This also prevents modernization or modification of the methods of their preparation or production, as there is no way to establish the equivalence of the product made by the modified method with the original product. Thus standardized drugs of well defined consistent quality are needed for reliable clinical trials and therapeutic use. The major reason advanced for the difficulty in developing quality control standards is that these products use whole plant or parts of plants or their total extracts, and in some cases even a mixture of a number of plants, it is thus challenging to develop suitable standards because a vegetable drug or a preparation thereof is regarded as one active ingredient in its entity, whether or not the constituents with therapeutic activity are known. Standardization of a herbal drug and of a preparation thereof is not just an analytical operation, it does not end with the identification and assay of an active principle rather it embodies total information and controls which are necessary to guarantee constancy of composition. Standardization of plant drugs has been stressed by the World Health Organization[78].

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*Recent Developments of Some Natural Products

M.B. Shah and G.Chauhan

L.M.College of Pharmacy, Ahmedabad

PLANT materials or their extracts have been utilised as drugs since long in many parts of the world, India and China being the oldest among them. In India formerly, only systems like *Ayurveda*, *Unani* and *Siddha* were found to be using plant materials in preparing dosage forms , but now other systems of medicine have also realised the importance of these products and manufacturing units preparing plant based drug formulations are found to be increasing to a large extent. However, in the industrialized countries, the isolation of active constituents and their incorporation in the dosage forms has gained much importance. In the developed countries active constituents derived from higher plants constituted about 25% of the prescribed medicines in 1989. Ecological awareness and an increased demand for nonclassical therapies may be invoked as the main reasons for this renewed interest in the plant based medicinal products. The present article deals with few reputed natural products derived from higher plants, majority of which are of Indian origin.

Monoterpene Iridoids

Bitter glycosides of monoterpene secoiridoids used as bitter tonic, stomachic, digestive and febrifuge are discussed here. Gentiopicroside, also known as Gentiapicrin and Gentiamarin is a characteristic bitter glycoside of Gentianaceae and is found in many species of Gentiana and Swertia. Gentiopicroside is a better crystalline water soluble glycoside with a bitter value[1] of 12,000. Sweroside found

*Revised and updated

in Swertia species is closely related to Gentiopicroside. Amarogentin is a trihydroxy diphenylcarbonic ester of Sweroside. Its bitter value[1] is 5,80,00,000. Amaroswerin (bitter value · 5,80,00,000) and Amaropanin (bitter value : 2,00,00,000) are also present in the drug. Picroside I and II and Kutkoside, present in subterranean parts of Indian Gentian (*Picrorhiza kurroa*) are C9 glycosides and contain epoxy oxide in the ring. Their structures are similar except that of trancinnamyl moiety in

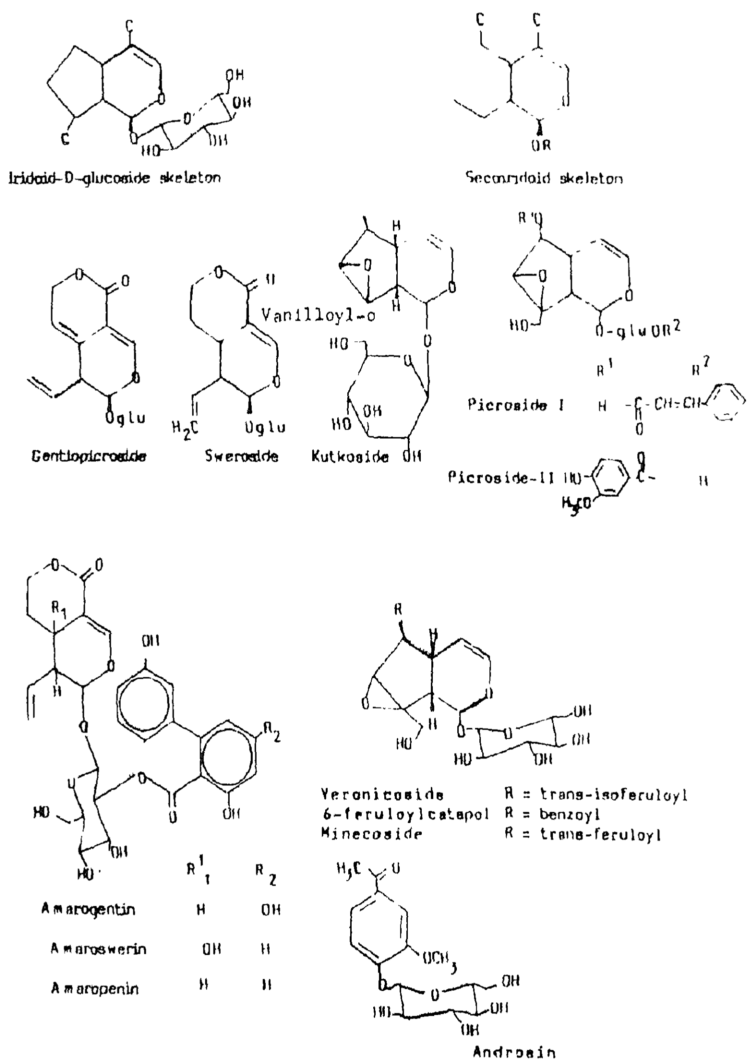


Fig.1

Picroside I and Vanilloyl in Picroside II and Kutkoside, are at different carbon atoms. Other iridoid glycosides, isolated from the roots are Minecoside, Veronicoside and 6-Feruloyl catalpol[2]. Immunomodulatory activity guided fractionation of aqueous extracts of *P. kurroa* reveals that Picroside II is much more potent than Picroside I. Apocyanin found along with these compounds, in fact exhibits the strongest activity[3]. The drug also possesses good antiasthmatic activity which is due to a phenol glycoside Androcin and it's aglycone Apocyanin. Chemical modification in these substances leads to increased activity[4,5,6] e.g., 4-hydroxy-3,5-dimethoxyacetophenone-Beta-D-glucopyranoside shows marked antiasthmatic activity. Moreover synthetic derivatives like Acetosyringenin and 2,4-dimethoxy-3-hydroxy acetophenone show more potent activity. An extract Picroliv containing Picroside I and Kutkoside as a major constituent in the proportion of 1·15 has been isolated from *P. kurroa* and is found to possess very good hepatoprotective and immunostimulant activity[7]. RLJ-NE 299A a standarised extract of *P. kurroa* consisting mixture of iridoid glycosides is shown to exert immunorestorative effect[8]. The drug is also found to have antileishmanial activity[7].

Valtrats

Valtrats, Valepotriats or Valtratums are the new group of epoxy iridoid esters isolated from fresh or stabilized roots of valerian. They are neither glycoside nor lactone and are said to be active primary components of the valerian. They are triesters of polyhydroxy cyclic pentanopyran, esterified with isovaleric, acetic, isocaproic and Beta-acetoxy valeric acid. They are about 0.5% in European valerian (Valeriana officinalis) and 2% in Indian valerian (*Valeriana wallichii*). They are classified into conjugated dienes: Valtrat and Acevaltrat giving blue colour with HCl and acetic acid, and monoenes : Didrovaltrat and Isovaleroxy hydroxydidrovaltrat (IVHD) giving yellow brown colour with HCl. Together with these compounds, a water soluble iridoid ester glycoside Valeriosidat without epoxy ring, is also found[9]. Valtrats are highly unstable compounds getting decomposed easily during drying into Baldrinal and free acids. They are used as tranquilizers and sedatives and their action is comparable to that of meprobamate. Like other sedatives, their effect is not synergistic with alcohol and barbiturates. Besides this, there is a quick disappearance of abstinaunce symptoms of alcohol and opium addicts. Large number of Valerian products, often in combination with other sedatives, are marketed, e.g. Valman, a well known formulation of Kali - Chemie from Hannover, contains Valtrat, Acevaltrat and Didrovaltrat in the proportion of 15:5:80. Recently, cytotoxic and anticancer activities of Valtrat and Didrovaltrat has also been discovered and Valtrat is said to be more potent in these actions than Didrovaltrat[10].

Valerian also contains other sedative and tranquilizing active constituents like Valeric acid and Valeranon. Japanese valerian (*V. angustifolia*) and Jatamansi (*Nardostachyas jatamansi*) are devoid of Valtrats and their sedative property, is said to be because of Valeric acid and Valeranon. It would be worthwhile to

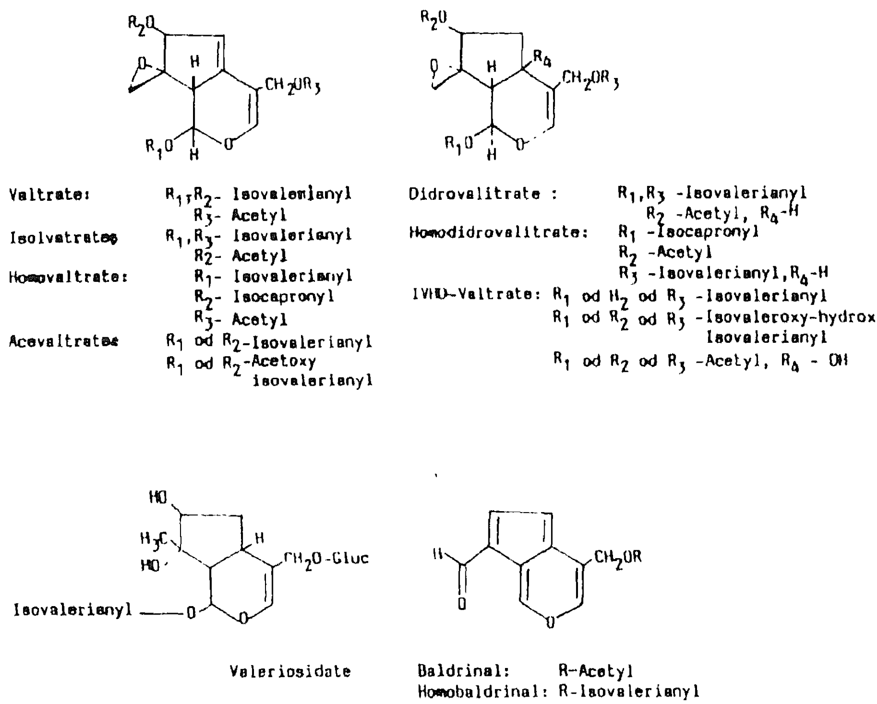


Fig:2

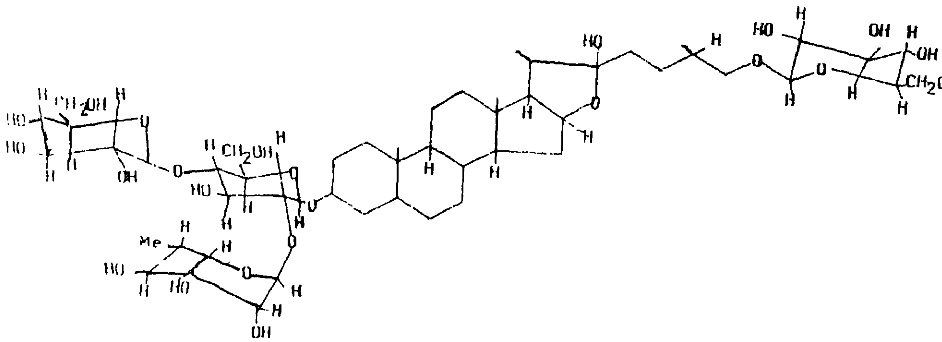
consider the manufacture of Valtrate in India from species like *V. wallichii, V. hardwickii*, etc.

Saponins

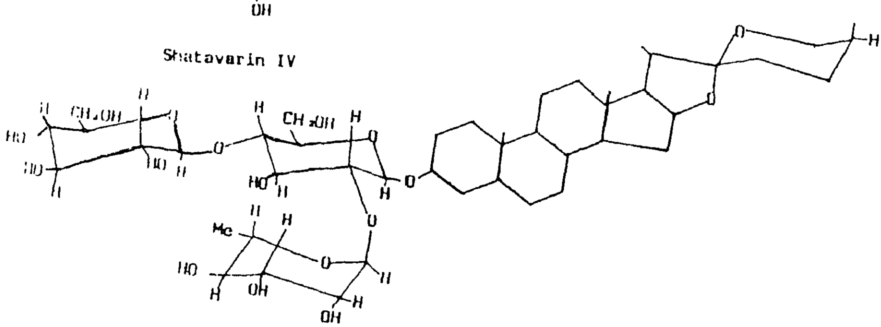
Saponins are glycosides characterized by foaming and haemolytic properties Chemically, they are classified into steroidal and triterpenoid saponins. They possess detergent, expectorant, diuretic, antitussive, antiinflammatory and antimicrobial actions. Drugs containing saponins are discussed below.

Sataver

It consists of tuberous roots of *Asparagus racemosus* (Liliaceae) Several therapeutic attributes have been mentioned in the classical Ayurvedic literature for this drug, which has been specially recommended as a galactogogue, antacid, tonic and in cases of threatened abortion. Recently its potent immunomodulatory activity has also been reported in animal models[11,12]. The market sample does not belong to this species but is either of *A. adscendens, A. gonoclads* or *A. officinalis*[13] It contains several steroidal saponin glycosides[14]. Shatavarin I - IV are the glycosides, I, II and IV are derived from a common aglycone moiety, Sarsaspogenin. The chemical

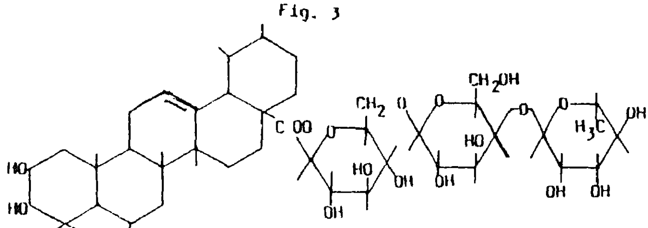


Shatavarin IV



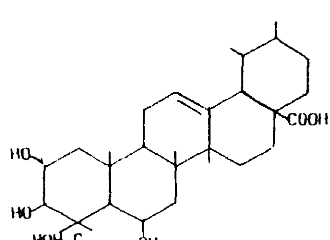
Shatavarin I

Fig. 3

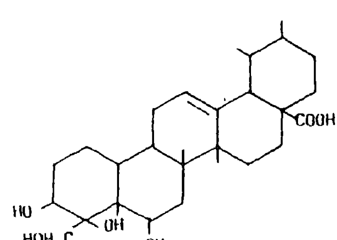


Asiaticoside R = H

Madecassoside R = β-OH



Brahmic acid



Isothankunic acid

Fig. 4

structure of Shatavarin IV has been elucidated[14] and is found to contain two glucose and one rhamnose. Shatavarin I is similar to Shatavarin IV but contains an additional glucose moiety. Shatavarin I possesses specific antioxytocic activity, both *in vitro* and *in vivo*. Chromosomal races have also been reported in *A. racemosus* containing sapogenins like Diosgenin and Sarsasapogenin in diploid, Sarsasapogenin in tetraploid and Diosgenin in hexaploid races[16].

Centella asiatica

Centella asiatica syn. *Hydrocotyl asiatica* (Umbelliferae) is a herb widely distributed throughout India. Its popular vernacular name is Brahmi - mandukparni or Mandukparni. It is highly reputed indigenous medicinal herb known for its adaptogenic property[17] and is recommended in cases of skin diseases, ulcers, for improving memory span and intelligence (brain tonic) and number of other ailments. Asiaticoside, Madecassoside, Brahmoside, Brahminoside, Indocentelloside, Thankunoside and Isothankunoside are the triterpenoid saponins isolated from the drug but the genetic variation is found to influence greatly the composition of these constituents in different ecotypes e.g. presence of Asiaticoside and Madecassoside in all but Brahmoside and Brahminoside in very few varieties[18]. Higher percentage of Asiaticoside (0.114) have been reported in plants growing in subtemperate Himalayan regions[19]. Recently isolation and structural elucidation of two new glycosides (Asiaticoside A and Asiaticoside B) have also been reported[20]

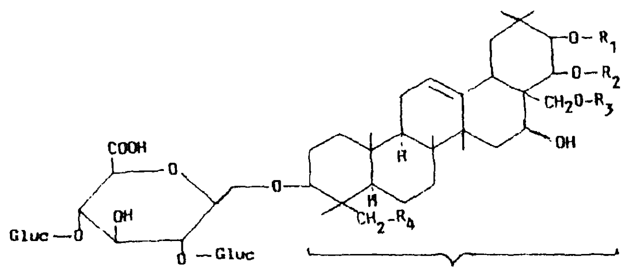
Pharmacological investigations of alcoholic and aqueous extracts of the plants reveal good antianxiety[21], antistress[22], antispasmodic[23], antiulcer[24,25], antifertility, CNS depressant[26] and narcotic-analgesic[27] properties. Number of its activities have been studied clinically also and the extract and Asiaticoside have been shown to possess very good antiulcer[28], antileproitic and wound healing properties. It has also been shown to improve the power of concentration and general ability and behaviour of mentally retarded children. Besides Asiaticoside other fractions of the drug are tested clinically and Madecassoside and Madecassoic acid are shown to possess antiinflammatory and wound healing properties while Isothankunoside, exhibits antifertility action. Patients treated with the drug also show improvement in varicose veins and other leg circulation problems[29]. In Brazil, it is used for uterine cancer and it has been shown to inhibit the growth of human cells *in vitro*[29]. The aqueous extract of the plant exhibits immunomodulatory activity[3].

Horse chestnut

Indian Horse Chestnut (Himalayan Chestnut) consists of the seeds of *Aesculus indica* (Hippocastanaceae). The seeds contain Aescin, a mixture of Beta-amyrin type saponins and is mainly composed of Protoaescigenin[30] and trisaccharide as a sugar moiety. Aescin possesses properties like galactogogue, wound healing, antiinflammatory, antimicrobial[31] molluscicidal[32], antiulcerogenic[33] and hypolipidaemic[34]. It is also used in cases of varicose veins, haemmorhoides, perivascular oedema, petechiac, skin diseases like eczema, psoriasis, contact dermatitis[35,36] and

in cardiovascular diseases[37]. Many of the venous effects of the drug are due to saponins which influence fluid exchange by reducing vascular permeability. Two new triterpenoid glycosides Aesculuside A[38] and Aesculuside B[39] are isolated from Aescin.

Various extracts of seeds and Aescin preparations of European species of Horse chestnut (*A. hippocastanum*) are patented[40,41,42] and are marketed in Europe for their antiinflammatory[43,44], antithrombotic[45] and antiexudative properties. Soft and dry extracts of the seed, Sterol/Aescin complex and Aescin are valued in cosmetic preparations[46,47]. Clinically, a formulation Topical Aescin 2% gel is reported to possess good effect in haematoma[48].



Protoaescigenin: R_1-R_2-R_3-H; R_4-OH

Barringtogenol: R_1-R_2-R_3-R_4-H

Aescin: R_1=Tiglyl, Angelyl, Isobutyryl, x-Methylbutyryl residues

R_2=acetyl; R_3=H; R_4=H or OH

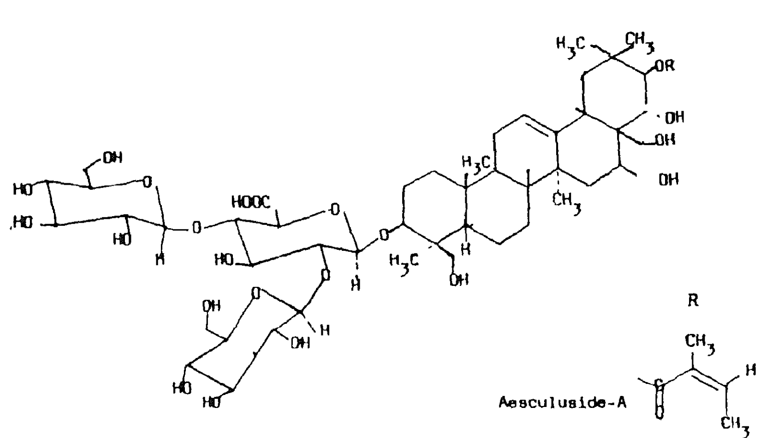


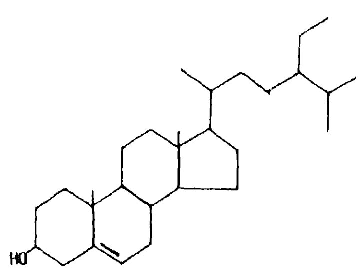
Fig.5

Sterols

Sterols are Triterpenes based on the Cyclopentanoperhydroxyphenanthrene ring system. Number of steroids are detected in plant tissues but Sitosterol, Stigmasterol and Campesterol are the three phytosterols commonly found is higher plants. They prevent the absorption of cholesterol from the G.I. tract. They are components of the unsaponifiable matter of the fixed oils and are obtained as a by-product of soap industries. Nowadays they are extensively used in the steroid synthesis, e.g. corn oil foots, abandoned as waste during oil refining, form an excellent substrate for direct fermentation of C-17 keto steroid intermediate for various steroid pharmaceuticals[49]

Sitosterol: (Beta-Sitosterol)

It is the most common sterol(Stigmast-5-en-3a-ol) a C29 compound, widely distributed throughout the plant kingdom, in free or in glycosidal form. Various seed oils like cotton seed oil, soyabean oil, corn oil, etc. are rich in Sitosterol. Sitosterol and drugs containing Sitosterol are found to possess hypocholesteraemic[50,51], antithrombotic[52], antiinflammatory[53], hypoglycaemic[54], antifertility[55] and spasmolytic activities. The antiinflammatory activity of Sitosterol from *Cyprus rotundus* is similar to hydrocortisone and oxyphenbutazone and the antipyretic activity is similar to acetylsalicyclic acid[57]. Besides this, unlike other antiinflammatory drugs, it possesses wide margin of safety[51] with minimum ulcerogenic activity[57]. In Germany, several formulations containing Sitosterol are used in the treatment of noninfective prostatitis and prostata adenoma. An antirheumatic formulation called Flemun containing Sitosterol is also marketed there by Intermuti, 3440 Eschwege.



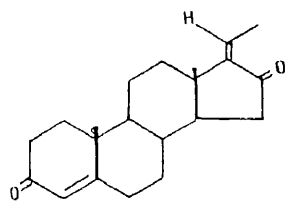
Sitosterol

Fig. 6

Guggul

It is the Oleogum resin exudate from the tree *Commifora mukul* growing wildly in the arid zones of India. The purified resin Suddha Guggul, Guggul Lipid (ethylacetate extract) and its polypharmaceutical preparations[58], like Kancanara Guggul, Kaisora Guggul, Triphala Guggul, Mahayograj Guggul, Laksa Guggul, Simhanade

Guggul, etc. are highly rated in the Ayurvedic Medicine for the treatment of number of diseases. Pharmacological and clinical investigations of all these products indicate that they possess properties like: antiarthritic[59,60,61], anticholesteraemic[62,63,64], antilipidaemic[62,64,65,66], antiobesity[67,68], antiinflammatory[60,65,69,70,71] and antiacne[72]. Platelet aggregation inhibitory action[73] and fibrinolytic action are also reported and the drug is frequently prescribed to patients with heart diseases[74,75]. It also possesses immunomodulatory activity[76].



Z-Guggulsterone

Fig. 7

Chemically Guggul is a complex mixture of Steroids, Diterpenoids, Aliphatic esters, Carbohydrates and varieties of inorganic ions The active constituents of the resins are present in the steroidal fraction, which yield sterols: Guggulusterols I, II and III; and sterones : Z Guggulusterone (1.6%) and E Gugglusterone (0 4%). Z Gugglusterone (Trans pregnandiene dione) shows good thyroid stimulating activity in rats. This thyroid stimulating activity[77,78] leads to reduction of serum cholesterol and serum lipids. Volatile oil of the resin shows good anthelmintic activity against tape worms and hook worms and it is better than piperazine phosphate and hexylresorcinol respectively[79]. Guggulip an antihyperlipidaemic formulation is marketed by Cipla in India and has also been promoted in the treatment of haemmorrhoids in combination with Vitexin as an antiinflammatory agent.

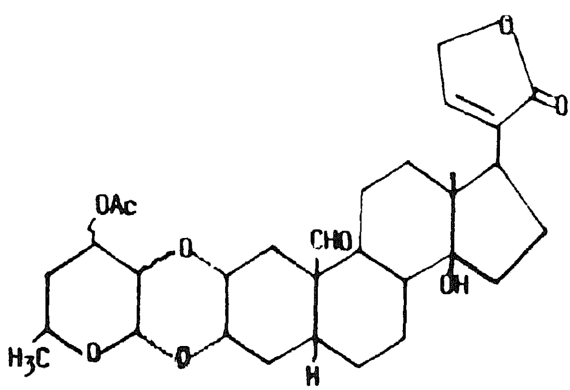
Cardenolides

Cardenolides are C-23 steroidal compounds exerting specific action on heart muscles. In plants they appear to be confined to the Angiosperms only and are particularly abundant in Apocynaceae and Asclepiadaceae. Thevatosides are amongst the most active of all cardiac glycosides.

Asclepias curassavica

A.curassavica is a perennial herb growing as a weed in arid zones of India. Indigenous system of medicine recommends the drug as purgative, emetic, a remedy for piles and gonorrhoea and in treatment of warts and cancer.

All parts of the plant contain potent cardiac glycosides but in the latex maximum amount which is highest (40%) amongst the Asclepias species is reported. The drug contains 22 cardenolides of which Calactin, Calotropin, Calotropagenin, Coroglaucigenin, Uzarin, Uzarigenin and Asclepin are isolated[80] The drug of Chinese origin contains a cardiac glycoside, Curassavicine which is more active than Digitoxin. Recently number of other cardenolides are isolated from various parts of the plant e.g. 3′-epi-19-norafroside and 12-beta-hydroxy coroglaucigenin(stem)[80], 16-alpha-hydroxy calotropagenin and 16-alpha-hydroxy calotropin and its 3′O-gentiobioside (seeds)[81], Voruscharin, Uscharin and Uscharidin(latex)[82]. Majority of these cardenolides are derived from Calotropagenin. The major therapeutic activity of the drug is due to Asclepin(0.011%) which is 3′-O-acetyl calotropin. The cardiotonic activity of Asclepin is more potent than Digoxin.



Aesclepin

Fig. 8

Besides this its action is quicker, more persistent, less cumulative and with wider margin of safety[83].

Thevetia neriifolia

Thevetia neriifolia syn. *Thevetia peruviana*(Apocynaceae) is a very common bush or a tree, distributed in tropical America, West Indies and India. It has been used in treatment of various skin diseases since the time of Charak. It possesses cardiotonic activity as well as insecticidal properties. The plant contains number of cardiac glycosides, sterols, iridoid glycosides and triterpenes. Although almost all parts contain cardenolides, maximum amount is present in latex and kernels. Thevetin A, Thevetin B, Neriifolin, Peruvoside, Ruvoside, Cerebrin(Monoacetyl neriifolin), Theveridoside, Thevefolin and Thevefoxin are isolated from seed kernels. While Neriifolin, Peruvoside, Neriifoside, Oleadoside, Nerioside, Alpha-L-rhamnosides of Digitoxigenin, Cannogenin and Thevetiogenin, the glycoside of

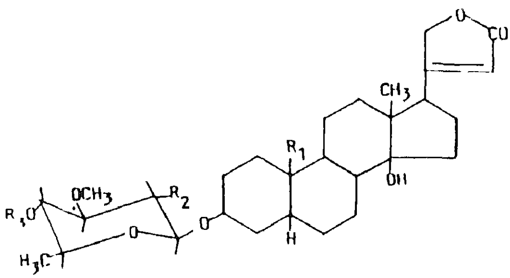


Fig. 9

	R_1	R_2	R_3
Thevetin A	CHO	H	Gentiobiosyl
Thevetin B	CH_3	H	Gentiobiosyl
Acetylthevetin B	CH_3	$COCH_3$	Gentiobiosyl
Thevebioside	CH_3	H	-D-glucosyl
Neriifolin	CH_3	H	H
Cerebrin	CH_3	$COCH_3$	H
Peruvoside	CHO	H	H
Ruvoside	CH_2OH	H	H
Thevefolin	CH_3	H	H

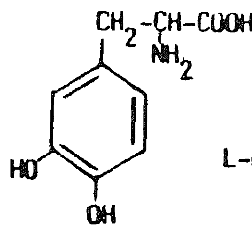
Uzarigenin, C-Nor-D-Homo-cardenolide-glycoside, Digitoxigenin-alpha-L-thevetioside and Alpha-L-acofrioside, two 18-20-epoxy derivatives of digitoxigenin, Alpha-L-thevetioside and 3-beta-O-(alpha-L-thevetose-3-beta-14-beta- dihydroxy-14(13-12) abeo- 5-beta, 12-beta, 14-beta-carda-13(18), 20(22)-dienolide from the leaves. Unlike the other cardenolides present in the plant which belong to Digitoxigenin series, Peruvoside, Ruvoside and Thevetin A belong to Cannogenin series. Peruvoside and Ruvoside are monosides while Thevetin A is trioside. The cardiotonic activity of the plant is due to Thevetin and Peruvoside. Peruvoside is Cannogenin-alpha-L- thevetoside possessing a very good cardiotonic activity. It is equipotent to Ouabain. It shows quick and powerful positive ionotropic effect, better absorption from G.I. tract, low cumulative toxicity and higher safety margin than Digoxin and Ouabain[83,84]. Clinical trials indicate that it is a promising drug for congestive cardiac failure. In Germany it is introduced under the trade name of Encordin.

Thevatia glycosides (Peruvoside, Theveneriin, Neriifolin) can be converted to digitoxigenin and digitoxose by enzymatic reaction and by selective 1,2-Beta hydroxylation to yield Digoxin. This process of conversion should be utilised on commercial scale in India to reduce the import of expensive cardiotonic drugs like Digoxin.

Amino Acid

Mucuna pruriens

M.pruriens, commonly known as "Kaunch" is a herbaceous twining annual found almost all over India. The ancient Ayurvedic system of medicine has described the medicinal use of the seed for the treatment of diseases of central nervous system and also as a geriatric tonic. The seeds contain about 5-6% of L.Dopa, 12.5% lecithins[85] and alkaloids. L-Dopa an amino acid (L-Dihydroxy phenylalanine) is a



L-Dopa

Fig. 10

highly reputed antiparkinsonian[86], fracture healing promotor[87] and antidepressant drug and its natural source is shown to give better protection and long term benefits as compared to synthetic Dopa[88]. Recently Zandu Pharm. Ltd., Bombay, has marketed a standardised processed seed powder of *M.pruriens* under the trade name of HP-200 for all sorts of parkinsonia. *Vicia faba, Mucuna semelvirens* and *Stizolobium cohinchinensis*[89] (3.34%) are some other leguminous seed drugs rich in L-Dopa, used in cases of Parkinsonia.

Pueraria tuberosa

P.tuberosa (Faboidae) is a perennial climber with large tuberous roots growing in the hilly regions of India. Dried sliced tubers are available in the market under the trade name of "Vidarikand". Tubers are sweet in taste and are used as supplementary food. The drug is popularly used in folk medicine as tonic, aphrodisiac, diuretic, cardiotonic, galactogogue, and also for birth control.

The tubers are rich in free amino acids[90], proteins and vitamin B1 Isoflavonoids like Puerarin, 4'-methoxypuerarin, 4'-6'-di-O- puerarin, Diadzein, Diadzin, Puerarone[91] and Neobava; Pterocarpans like Tuberosin, Deoxytuberosin and Hydroxy-tuberosone[92] and a coumestan Puerarostan[91] are isolated from the roots. Tuberosin shows anti-staphylococcal, antitubercular and antifungal activities, while Puerarin shows good protective effect on the ischaemic myocardium[93,94] Butanol extract of the root exhibits significant antihepatotoxic activity[95]. Pharma-

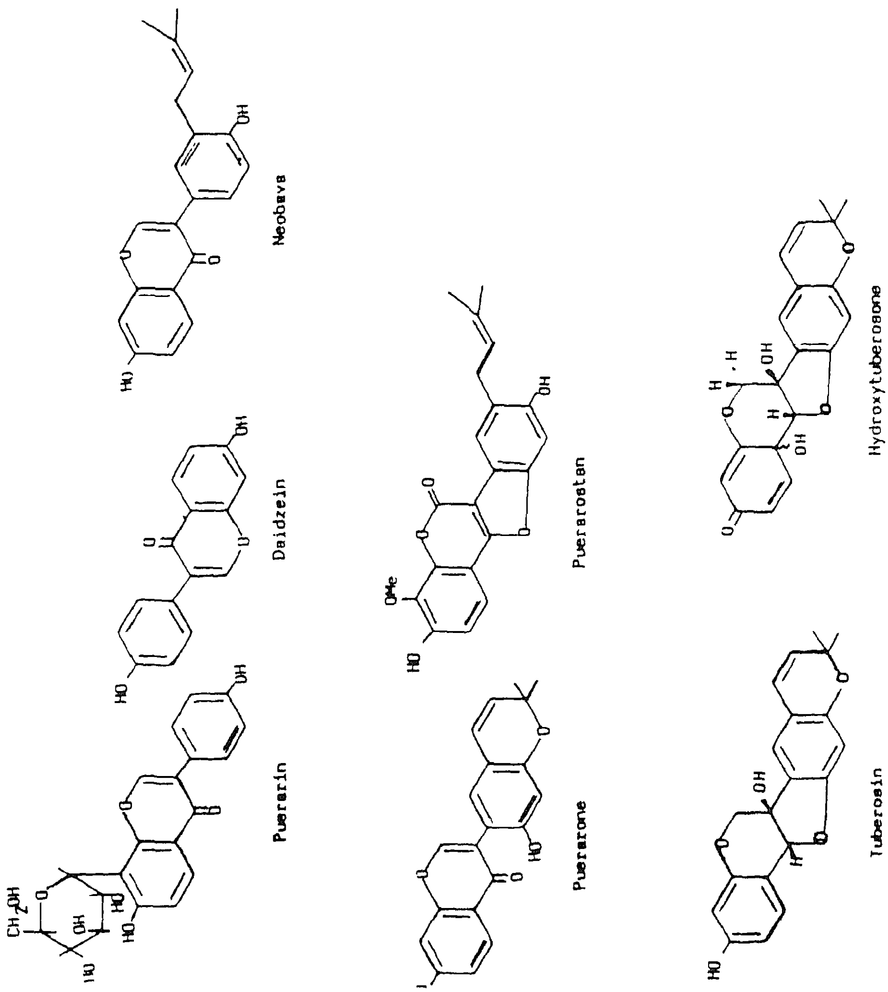

Neobava
Daidzein
Puerarin
Puerarostan
Puerarone
Hydroxytuberosone
Tuberosin

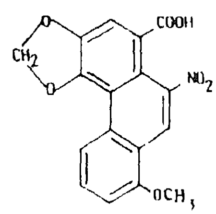
Fig. 11

cological studies with butanol, chloroform and ethanol extracts of roots indicate very good estrogenic[96], antiimplantation[97,98,99] and antifertility[97,100,101,102] activities, the butanol extract being the most potent and without any side effects.

Alkaloids

Aristolochia indica

A.indica (Aristolochiaceae) is a perennial climber growing all over India. Roots and rhizomes parts of the plant are reputed in indigenous system of medicine for its gastric stimulant and bitter tonic drugs. *A.bracteata* is another Indian species which is used as a substitute of *A.indica*. It is commonly known as "Kidamari" and is used as bitter tonic, purgative and anthelmintic.



Aristolochia acid

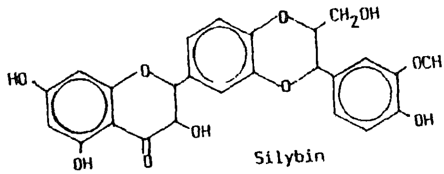
Fig. 12

Roots of *A.indica* contain Aristolochic acids[103], Aristolactams, Aristolochine, sesquiterpenes, steroids and p-Coumaric acid. An alkaloid Aristolochic acid - the chief active principle of the drug is 3-4-methylenedioxy-,8-methoxy-10-nitro-1-phenanthrene carboxylic acid. It is intensely bitter substance and is highly valued for its anti-fertility[104,105], antiimplantation and abortifacient properties. Aristolochic acid is found to be good contraceptive for both males and females[106]. It also exhibits antitubercular, antiviral, antifeedant and phagocytosis stimulating properties. Earlier reports indicate it to possess antineoplastic[107] and antiinflammatory activities but later on it has been proved to be carcinogenic and mutagenic.

Flavonoids

Silybum marianum

Silybium marianum (previously *Cardus marianum*) (Compositae), commonly known as "Milk Thistle" is an annual or biennial plant growing as a weed in Kashmir, Punjab and other northwest parts of India, USA and Australia. The herb is also cultivated and traditionally used as a bitter digestive, liver tonic and poison antidote. German physician Rademacher reported success in giving it to liver

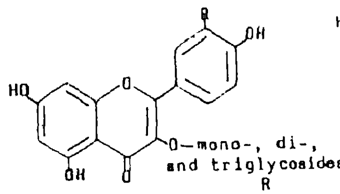


Silybin

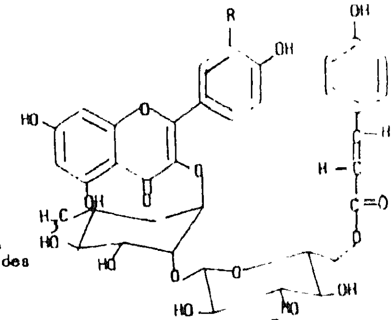
Fig. 13

patients in the early 19th century. Fruits are used in medicine and contain number of flavonoligans such as Silymarin, Silyin, Silydianin, Silychristin, Silybinin, Silymonin, 3- deoxysilychristin and Silandrin. It also contains flavonoids like Naringenin, Eridictyol, Apigenin, Chrysoeriol, Apigenin-7-O-beta- (2"-O-alpha-rhamnosyl) galacturonide, Apigenin-7-O-beta-glucuronide, 6′-ethylester, Apigenin-7-O-beta-glucoside, Apigenin-7-O-beta-galactoside, Kaempferol and Taxifolin. Other constituents reported in the drug are alkaloids, saponins, phytosterols, tannins and fixed oils. The remarkable hepatoprotective activity of the drug is due to flavonolignans[108] especially Silymarin and Silybin; the former is located in the thick-walled cells of the seed epidermis[109]. Silymarin is a potent hepatoprotective[110,111,112,113] agent and is used as standard for evaluating herbal hepatoprotective drugs. Silybin when injected to human patients upto 48hrs. after they accidently ingested deathcap mushroom (*Amanita phalloides*) which contains most potent liver toxins known, it prevented the normally anticipated fatalities[114]. Many commercial preparations are manufactured in Germany from the fruits. An official Tinctura Cardui Mariae Rademacher is still listed in the Pharmacopoeias of some countries. An extract from the aerial parts of *S.marianum* containing flavonolignans is patented[115]. Silipide[116,117,118] a complex of Silybin with Phosphatidylcholine shows higher lipid solubility and hence is more active than Silybin in liver damage. Silybindihemisuccinate[119,120], a soluble isomer of Silymarin obtained from the drug acts as an antidote against acetaminophenone hepatotoxicity. Clinical studies show that symptoms of acute hepatitis, especially digestive problems, improved within two weeks after taking Milk thistle. Well being and appetite also improved. It has successfully treated patients with chronic hepatitis and cirrhosis of the liver. The detrimental effects of jaundice, drugs, environmental toxins and alcohol on the liver is encountered with the drug.

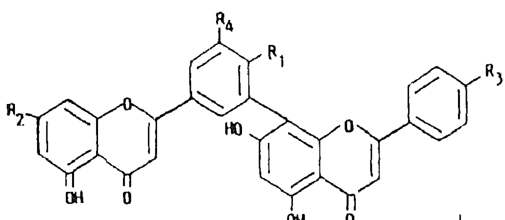
Besides hepatoprotective activity, number of other activities have been reported for Silymarin e.g., membrane stabilizing[121], usefulness in vascular disorders, antioxidant[122], cytoprotective[123] and potassium sparing diuretic activity[123]. Silymarin-phospholipid combination (Silymarin-phytosome) possesses antiwrinkle activity controlling ageing of the skin[124]. Silicynar, obtained from the drug is proved



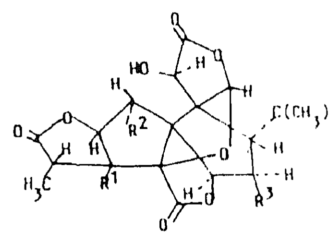
	R
Kaempferol derivatives	H
Quercetin derivatives	OH
Isorhamnetin derivatives	OCH_3



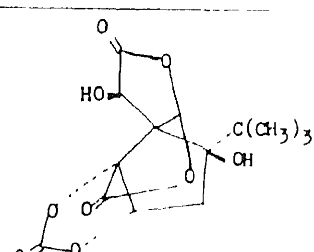
	R
Kaempferol derivative	H
Quercetin derivative	OH



	R^1	R^2	R^3	R^4
Ametoflavone	H	H	H	H
Bilobetin	OCH_2	OH	OH	H
Ginkgetin	OCH_3	OCH_3	OH	H
Isoginkgetin	OCH_3	OH	OCH_3	H
Sciadopitysin	OCH_3	OCH_3	OCH_3	H
5'-methoxybilobetin	OCH_3	OH	OH	OCH_3



Ginkgolide	R_1	R_2	R_3
A	OH	H	H
B	OH	OH	H
C	OH	OH	OH
J	OH	H	OH
M	H	OH	OH



Bilobalide

Fig.14

to the effective in patients with chronic pancreatitis[125]. Three chemotypes of *S.marianum* according to flavonolignan patterns in the fruits have been reported[126].

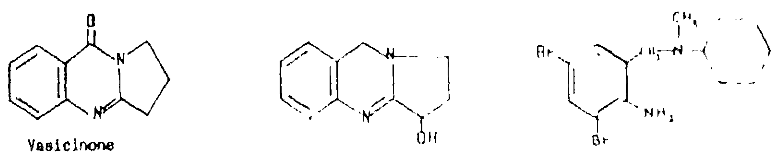
Gingko biloba

Gingko biloba(Gingkoaceae) is a large perennial tree indigenous to China. It is the only surviving species of Gingkoales (Gymnosperm) which evovled about 240 million years ago and is considered as a "Living fossil" by Darwin. It is cultivated in China, Japan, Europe and occasionally in hilly regions of India. It is a promising drug plant having history of being used for various diseases appearing with old age, like reduced mental and physical efficiency, loss of concentration, memory impulsion, vertigo, vigilance, etc. It enhances memory by increasing blood flow, metabolism efficiency, regulating neurotransmitters and boosting oxygen levels in the brain and hence it is gaining recognition as a brain tonic drug. It is also valued for it's cosmetic use[36]. Gingko leaves contain long chain hydrocarbons and derivatives like Polyprenyl[127,128,129,130,131] and Polyprenyl acetate[132], acids like Anacardic acid, Carbohydrates and derivatives Flavonoids, Isoprenoids(sterols, terpenoids), various compounds like (Z-Z')-4,4'-(1-4pentadiene-1,5-diyl)-diphenol, Catechins, Proanthocyanidins, Cytokinins, beta-lectins, Carotenoids and others. Among the main known flavonoids a great variety of flavonol glycosides based on Kaempferol and Quercetin occur as mono- , di- and triglycosides like Astragalin, Rutin and number of other glycosides of Kaempferol and Quercetin[132]. Minor flavonoids include compounds derived from Isorhamnetin, Myrecetin, 3'- methylmyricetin, Apigenin and Luteolin. Moreover leaves contain flavonoid glycoside esters with Courmaric acid[132]. Non-glycoside biflavonoids isolate from the leaves include Amentoflavone, Ginkgetin, Isoginkgetin, Bilobetin, Sciadopitysin and 5'-methoxybilobetin. The triterpenoids isolated from the leaves (0.266%) are highest in autumn. The activity of the drug is due to Ginkgolides and Bilobalide. Pharmacological and clinical studies have indicated it's use in cases of peripheral circulatory insufficiency[133,134], due to degenerative angiopathy and cerebrovascular insufficiency with symptoms of vertigo, tinnitus, headache, dementia, vigilance, mood disturbances, hearing loss [135] and in geriatric conditions[136,137]. The extract antagonises platelet aggregation induced by platelet activating factor[138,139] and the four compounds responsible for this are Gingkolides A,B,C and J[140] but the activity is limited by poor bioavailability. Gingkolide B(BN-52063) acts as potent platelet activating factor antagonist[141], besides this it also inhibits thrombus formation *in vivo* and produces thrombolysis. In various models of cerebral ischaemia, Gingkolides exhibit hypoxia protective activity[134] as well as reduction in post ischaemic lesions. The extract inhibits immunocomplexes induced pancreatic oedema.[142], The Gingkolides have shown to subdue allergic inflammation, anaphylatic shock and asthma[143,144]. Both Gingkolides and Bilobalides increase blood flow in patients with cerebrovascular diseases. Bilobalide causes regeneration of motor nerves[145]. Biflavonoids are active in peripheral vasodilation[145] and also exhibit antibradykinin activity[146]. In Europe Gingko phytoformulations based on these extracts are well

known and marketed under the names of "Tebonin", "Rokan", "Tanakan", "Gingk obil" etc. Gincosan(PHL 00701)[147,148] a combination product of standardised extract of *G. biloba* (GK501) and *P. ginseng* extract (G115) shows improved retention of learned behaviour.

Adhatoda Vasica

Adhatoda vasica (Acanthaceae), a perennial shrub is distributed throughout India It has been recommended for various ailments of respiratory system like cough, bronchitis and bronchial asthma in the indigenous system of medicine Number of proprietary expectorant formulations are marketed in the country It is also a reputed haemostatic antihaemorrhagic drug of Ayurveda. It contains number of Quinazoline alkaloids like Vasicine, Vasicinone, Anisotine, Vasicinol, Deoxyvasicine,



Vasicinone Vasicine Bromhexine

Fig. 15

Vasicinine, Vasicoline, Vasicolinone, Adhatodine and 1,2,3,9-tetrahydroxy-5 methoxy quinazoline; steroidal compound Vasakin, Bromohexine and it's derivatives like BR-227, beta-sitosterol, Volatile oil, Aliphatic alcohol, Ketone Lignins, Resins, etc. The total alkaloidal and Vasicine content[149] is found to be highest (2.20% and 2.09%) in August and lowest (0 70 and 0 35) in February Vasicine is bitter and gets racemized during the isolation process. Vasicine and Vasicinone show bronchodilatory activity comparable to that of Theophylline and an individual alkaloid exerts greater activity[150]. Recently it has been proved that Vasicine and Vasicinone are devoid of any anti-asthmatic activity[151,152] but a minor alkaloid Vasicinol(inhalative) exhibits significant activity[153] Bromhexine and it's derivatives also possess expectorant activity[154]. Vasicine exhibits moderate hypotensive and cardiac depressant activities while Vasicinone is devoid of these activities Vasicine possesses strong and extremely selective uterine stimulant activity and is a promising uterotonic abortificient[155,156]. It is also useful for the control of postpartum haemorrhage[157]. The drug also shows antifungal, juvenomimetic[158], antifeedant[159,160], insecticidal and repellant activities. It possesses antiinflammatory properties[161] and is effective in gingival inflammation[162] and pyorrhoea[163]

Miscellaneous

Allium cepa (Onion)

Allium cepa (Liliaceae) is a bulbous biennial, cultivated throughout Indian subcontinent. Bulb is used for culinary, vegetable and medicinal purposes It is used as rubifacient, poultice for boils and abcesses, tonic, diuretic, stomachic, stimulant

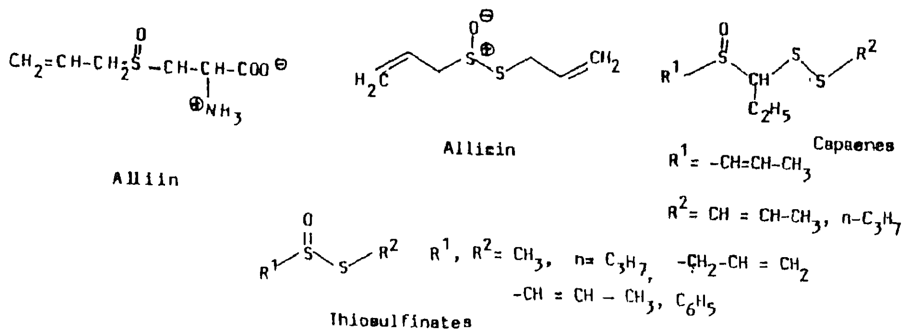


Fig. 16

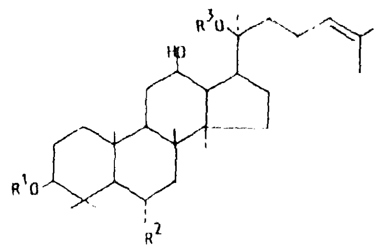
and expectorant. It contains Amino acids (Alliin[164] and Allicin[165]), Proteins, Carbohydrates, Vitamin C, Volatile oil (0.005%), Phenolic acids, Flavonoids. Anthocyanins, Oleanolic acid, Diphenylamine[166], Steroidal glycosides[167,168], Alliospiroside A, Alliospiroside B, Alliofuroside and Phytoalexins[169]. The medicinal activity of the drug is attributed to it's oil content. It contains Thiosulfinates[170,171,172,173], Dithietanes[170,173] and Alphasufinyl disulfides[170,172,174] (Capaenes). The oil and its individual constituents have been studied pharmacologically and clinically and are shown to possess number of properties like hypoglycaemic[175,176] (Diphenylamine), aphrodisiac[177], hypo cholestraemic[178,179,180], platelet aggregation inhibitor[174, 180,181,182,183,184,185] (1- methyl-(sulfinyl)- propyl- methyl- disulphide), antiasthmatic[172,174,186,187] (Alpha- and Beta- unsaturated thiosulfinates and Thiosulfinates and Capaenes; antifungal[171], antimicrobial[188] (Steroidal glycosides), abortive[189], juvenoid[190], antiartherosclerotic[191] and immunosuppressive[192].

Adaptogens

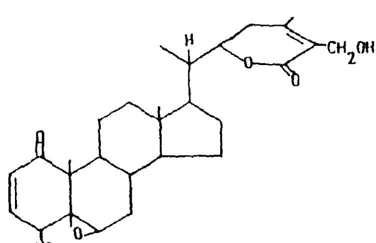
Ginseng

Panax ginseng (Araliaceae) is a perennial herb growing as a weed in countries like Korea, China (Panax ginseng) and Himalayan regions of India (Pseudoginseng). Tibetan system of medicine mentions the use of the drug since 5000 years. Modern research shows that is possesses exceptional medicinal properties. All parts of the plant contain active principles but roots are rich in saponins and possess number of important properties like adaptogenic, antistress, immunostimulant, etc. Adaptogenic activity of the drug is attributed to Saponins termed Ginsenosides and Panaxosides. Indian pseudoginseng contains saponins like Ginsenosides (R0, Rb1, Rb2, Rb3, Rc, Re, Rd, Rg and Rg1), Chikusetsusaponins(IV, IVa and V) and Pseudoginsenosides (RI1, RI2, RI3, RP1, RT1 and F11). Saponins isolated from Panax ginseng are Ginsenoside R0, Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, RGL, Rh1, Rh2 and La. Ginsenosides belong to two types of Sapogenins, 20S-protopanaxdiol

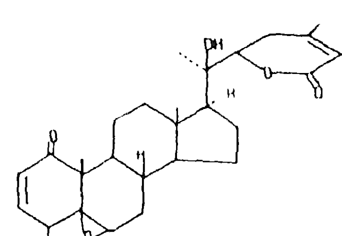
and 20S-protopanaxtriol. The Panaxosides A,B,C etc. upon hydrolysis yield mainly Oleanolic acid, Panaxdiol and Panaxtriol. Besides Saponins the drug contains Polysaccharides, Panaxans and Alkaloids. Ginsenosides are having complex metabolic and physiological properties. Acute administration of individual purified Ginsenosides or alcohol extracts of the drug results in stimulation of hepatic protein synthesis leading to increased ribosomal number and RNA-activity as well as carbohydrate intermediatory metabolism in liver, adipose tissue and skeleton muscle. These actions are most prominent during stressful conditions or altered



Compound	R¹	R²	R³
(20S)-Protopanaxadiol	H	H	H
(20S)-Protopanaxatriol	H	OH	H
Ginsenoside R_{a-1}	Glc-²Glc	H	Xyl-⁴Ara(p)-⁶Glc
R_{a-2}	Glc-²Glc	H	Xyl-²Ara(f)-⁶Glc
R_{b-1}	Glc-²Glc	H	Glc-⁶Glc
R_{b-2}	Glc-²Glc	H	Ara(p)-⁶Glc
R_{b-3}	Glc-²Glc	H	Xyl-⁶Glc
R_c	Glc-²Glc	H	Ara(f)-⁶Glc
R_d	Glc-²Glc-	H	Glc
R_e	H	Rha-²Glc-O-	Glc
R_f	H	Glc-²Glc-O-	H
R_{g-1}	H	Glc-O-	Glc
R_{g-2}	H	Rha-²Glc-O-	H
20-Gluco-R_f	H	Glc-²Glc-O-	Glc
R_{h-1}	H	Glc-O-	H



Withaferin A



withanolide D

Fig. 17

nutritional states. It acts as an antistress by conserving endogenous body carbohydrates[193,194].

Ginsenosides(Rg1 Rh1 Rb2) have definite *in vitro* effect on central neurotransmitter receptors and protein biosynthesis[195]. Ginseng extract inhibits protein degradation and stimulates protein synthesis in human fibroblasts[196]. Ginsenosides improve blood lipid metabolism and thereby prevent artherosclerosis, moreover they show inhibition of Ca^{++}- effluence from bone when tested on animal model[197]. Ginsenoside Rg1, Rg2, Rg3, Rh1, Rh2 and R0 and Panaxynol show inhibition of platelet aggregation *in vitro*[198]. Ginsenosides have definite effect on myocardial structure[199,200,201] and are proved to be efficacious in cardiovascular diseases (increase the blood flow and decrease the oxygen consumption). Ginsenosides Rg3, Rh1, Rh2 and R0 are found effective in experimentally induced disseminated intravascular coagulation[202,203]. Ginsenosides (Rg1 and Rb1) and standardised ginseng extract (G115) exhibit antilipidaemic activity[204,205] (supresses cholesterogenesis). Ginseng is shown to exert profound hypoglycaemic[206,207,208], antipholigistic (Ginsenoside R0)[197], antihepatitic (Ginsenoside R0)[209] and antiulcer activities[210,211]. Saponins and std. extr. (G115) antagonise narcotic effect of morphine[212,213]. Panaxytriol possesses significant antitumour activity[214,215]. Moreover the drug potentiates the antitumour action of Mitomycin C [216,217,218]. It acts as an immunostimulant[219], Ginsenosides and Panaxytriol[215] promote the function of immunological function cells[220]. Besides this soluble polysaccharide fraction (heteroglycan) exhibits significant anticomplementary activity [221]. The protein fraction of Ginseng shows significant radioprotective effect. The lipid soluble fraction of the drug exerts a stimulatory effect on a paraneuronal culture cell line promoting neurite outgrowth indicating usefulness of the drug in the treatment of dementia with progressive degeneration of brain neurons[222,223]. The drug is also valued as a cosmetic[224,225].

Ginseng induces corticosterone secretion and has been described to produce corticoid like syndrome causing insomnia, oedema, hypertension and withrawal symptoms(restlessness and diarrhoea).

Withania somnifera (Ashwagandha)

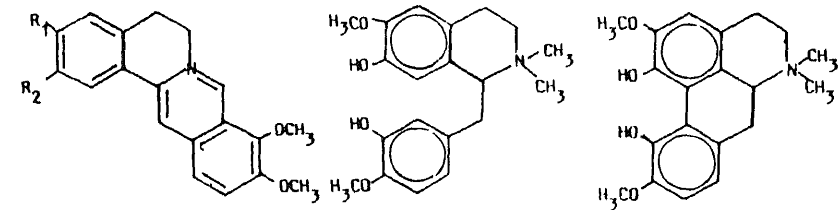
Withania somnifera (Solanaceae), is an evergreen tomentose perennial shrub growing as a weed throughout the arid zones of India. Root parts contain higher percentage of alkaloid and are collected from cultivated plants also. High yield of roots and alkaloids (0.27%) has been obtained from a newly developed cultivated variety "WS-20" of Withania[226]. Ayurveda categorises the drug as "Rasayana" and mentions number of its uses like nervine tonic, sedative in cases of insomnia, epilepsy and hypertension, anodyne, diuretic, etc., but it is the adaptogenic property which has made the drug more reputed recently[227,228].

The pharmacological activity of the drug (roots and leaves) is attributed to Alkaloids and Withanolides. The roots contain number of Tropane alkaloids,

Cuscohygrine, Anahygrine, Tropine, Pseudotropine, Nicotine, Somniferine, Somniferinine, Withaninine, Withanine, Pseudowithaninine, Choline, Anaferine, Withasomine, Visamine, Withaniol (mixture of two withanolides), Withanolides like Withaferine A, Withanolide N,O,D,P,S,Q,R,G,H,I,J,K,V, Sitoindosides IX,VII and VII, a Withanolide 5,2-O-Alpha(R)-dihydroxy-6alpha-7alpha-epoxy-1-oxo-(15A lpha)-witha-2,24-dienolide, a new withanolide Withasomidienone-27-OH-3-oxo-(22R)-witha-1,4,24-trienolide[229] and Solasodine. Besides these the drug contains Volatile oil, Reducing sugar, Starch, Amino acids, etc. Pharmacological and clinical studies denote the drug as antistress[230], analgesic[231,232], antiinflammatory[233,234], antitumour[235,236], antibiotic, anticonvulsant[237], antihepatotoxic[238,239], psychotropic, hypotensive, antispasmodic[240], and CNS depressant. The drug is highly safe[241], the LD50 value being 1260 mg/kg. Withanolides have been shown to possess both immunosupressive and immunomodulatory properties[242], the later being more specific to Withanolide E[243]. The Sitoindosides VII, VIII and Withaferin A, in combination exert antistress activity[244]. Sitoindosides IX and X are found to possess adaptogenic and immunostimulatory activity[245,246]. A steroidal lactone 5,20-(R)-di-OH-6,7-alpha epoxy-1-oxo-(5-alpha) witha-2,24-dienolide and Solasodine isolated from the drug also exhibit adaptogenic and immunomodulating activity[247]. Withanolides, possess antiinflammatory and antiarthritic activity[234] against various models of inflammation[233,234,248]. The antitumour activity of the drug is attributed to Withaferin A[249] and Withanolide D[250]. Withaferin A (found in roots and leaves) shows the activity due to the presence of unsaturated lactone ring in the side chain with a allylic primary alcohol group at C25 and highly oxygenated rings at the other end of the molecule. Further the drug is reported to increase effect of radiation on tumour regression as well as growth delay[251]. Withaferin A has shown potential as an antibiotic also. The extract of the drug exhibits good anticonvulsant[237,252] activity. It is also having CNS-depressant effect and it's role in anaesthesia is reported[253]. Number of herbal formulations containing *W.somnifera* as one of the active ingredients are marketed in India. These formulations are evaluated and are shown to possess good analgesic, antiinflammatory[254], antiarthritic[255], lithnotriptic and nervine stimulating properties[256].

Tinospora cordifolia (Guduchi Giloe)

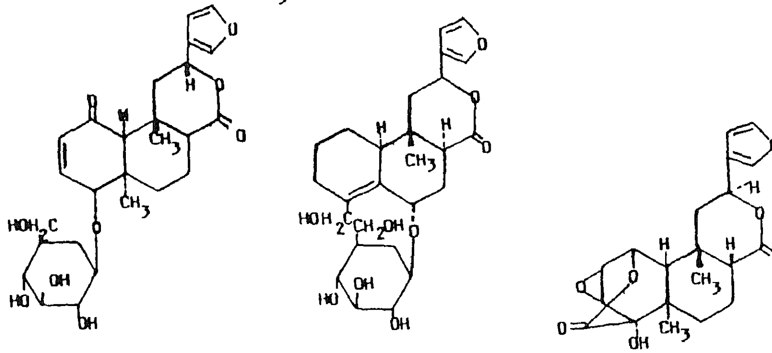
T. cordifolia (Menispermaceae), is a perennial climber, distributed throughout the tropical Indian subcontinent. It is categorised as Rasayana in Ayurveda and is well known for it's adaptogenic[227] and immunomodulatory activity in fighting infections. Stem is used as a general tonic, antiinflammatory, antiarthritic, antimalarial, antidiabetic, antiallergic and aphrodisiac. It is also useful in skin diseases, anaemia, piles and chronic fever. "Guduchi satwa" (an aqueous extract containing starch) is a nutrient and is used in chronic diarrhoea and dysentry. Juice prepared from fresh plant is used as a diuretic. The roots are used for its antistress, antileprotic and antimalarial activities. Alkaloids belonging to isoquinoline group viz. Berberine, Palmatine, Tembetarine, Mangoflorine, Choline and Tinosporin and Glycosides, 18-norclerodone glucoside and Furanoid diterpene glucosides viz. Cordifolisides



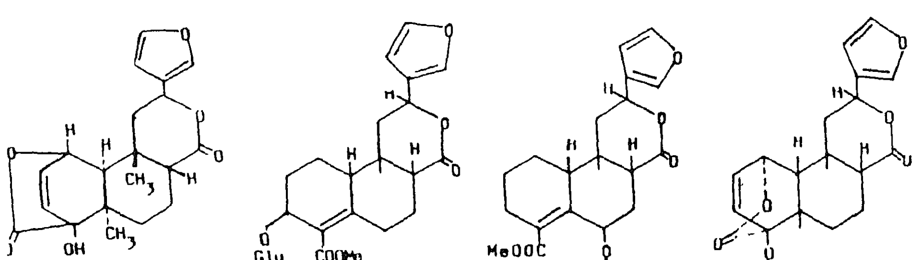
Berberine R^1,R^2= O-CH-O
Palmatine $R^1=R^2=OCH_3$

Tembetarine

Magnoflorine



18-norclerodane glucoside. Furanoid diterpeneglycoside. Tinosporide.



Columbin

Cordifoliside A B

Cordifoliside C

Cordifoliside D

2,3-epoxy Cordifoliside E

Fig. 18

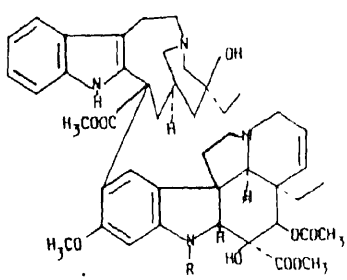
A-E and two phenylpropane have been isolated from the stem. Other compounds reported in the plant are Sterols, a phenolic lignin, Tinosporidine, Cordifol, Cordifelone, Lactones like a Diterpenoid furanolactone, a Clerodane derivative, Tinosporon, Tinosporide and Columbin. Starch is mainly composed of 1,4-linked glucan with occasional branching. The activity of the drug appears to be due to alkaloids. The drug shows immunomodulatory activity[257,258,259,260,261]; it is shown to be effective against various types experimentally induced infections[262]. It is reported that it produces significant leucocytosis and predominant neutrophilia in animal models. Clinical experiments for its anti-stress and tonic properties subdue its use in cases of mentally retarded children [263,264]. The aqueous or alcoholic extract of plant and leaf exhibits hypoglycaemic and antihyperglycaemic activities in

different experimental models[265]. A Pyrrolidine derivative isolated from ethyl-acetate exhibits hypoglycaemic activity in animal models[266]. The aqueous extract is also reported to exert a significant antiinflammatory effect which is comparable with Indomethacin and the mechanism of action appears to resemble non-steroidal antiinflammatory agents. The drug possesses significant hepatoprotective activity[267,268]. The drug reveals other activities like antibacterial (*M.tuberculosis*), antipyretic[269], hypotensive, diuretic, etc. Herbal formulations Rumalaya[270] and Septillin[271] containing *T.cordifolia* upon clinical evaluation are shown to be effective in rheumatoid arthritis and in children suffering from respiratory tract infections respectively. Septillin is shown to have immunostimulatory effect when tested in animals bearing tumours[272].

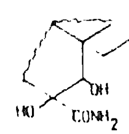
Anticancer Drugs

Vinca Alkaloids

The first most successful phytoconstituents used in cancer chemotherapy are the alkaloids of *Catharanthus roseus* (Apocynaceae) commonly known as "Periwinkle". Intense work on Madagascar Periwinkle, a folklore hypoglycemic drug led to the isolation of a number of Dimeric indole alkaloids having antineoplastic activity. Two of these alkaloids Vinblastine (Vincaleukoblastin) and Vincristine(Leurocristin), present in minor quantities(0.0002%) are now commerically extracted from *C.roseus*. Velban(Vinblastin sulfate) and Oncovin (Vincristine sulfate) are marketed by Eli Lily as anticancer drugs. Cipla labs has improved the process of isolating Vinblastine and Vincristine from *C.roseus* developed by NCL, Pune Today India is the third largest manufacturer of Vinblastine and Vincristine in the world and is exporting these alkaloids to European countries



Vinblastine R = CH_3
Vincristine, R = CHO



Vindesine R = CH_3

Fig. 19

Although structurally closely related, Vinblastine and Vincristine exhibit significant difference in their clinical utility and toxicity.Vinblastine is useful in treatment of patients with Hodgkin's diseases, non-Hodgkin's lymphomas and renal, testicular, head and neck cancer. Vincristine is widely used in combination

with other anticancer agents, in the treatment of acute lymphocytic leukaemia in childhood and for certain lymphomas and sarcomas, small cell lung cancer, cervical and breast cancer[273]. These compounds are antimitotics inhibiting cell growth, at least in part, by disrupting microtubules, causing the dissolution of cell mitotic spindles and the arrest of cells at metaphase[274]. High demand and low yield of these alkaloids in the plant, has led to search for alternative means for their production. Nowadays Vinblastine is converted into Vincristine either chemically or via microbiological N-demethylation using Streptomyces albogriseolus. Vinblastine is also modified structurally to yield Deacetyl vinblastine amide (Vindesine), introduced recently as Eldisine for use in the treatment of acute lymphoid leukaemia in children. A Spirolactone (4-dioxolanone) derived from 17-deacetyl-vinblastine, exhibits antitumour activity[275]. Biochemical coupling of alkaloids Catharanthine and Vindoline to get dimeric compounds is also achieved[276,277]. Alpha-3',4'-anhydrovinblastine, a precursor of Vinblastine and Vincristine can be enzymatically synthesized *in vitro*[278]. A structural variant 2,5'-noranhydrovinblastine is developed as an anticancer drug and has been introduced in France recently. Besides these, tissue culture technique is developed for the production of these dimeric alkaloids[279,280].

Podophyllotoxin

Podophyllotoxin, an Aryltetralin lignan, is an anticancer principle of Podophyllum species (Podophyllaceae) contained in resin podophyllin. Podophyllin is obtained from rhizomes of *P. hexandrum* or *P .emodi* (Indian podophyllum) and *P. peltatum*(American podophyllum). Indian podophyllum yields 6-12% of Podophyllin the amount being highest in May (flowering) and is free from Peltatins, while the American drug contains only 2-8% of Podophyllin and alpha- and beta-peltatins. *Linum album* is also a good source of Podophyllotoxin. 5-methoxy-podophyllotoxin-4-beta- glucoside found in root cultures of *Linum flavum* exhibits cytotoxicity and the aglycone has 250-500 times more activity[281]. Podophyllotoxin is

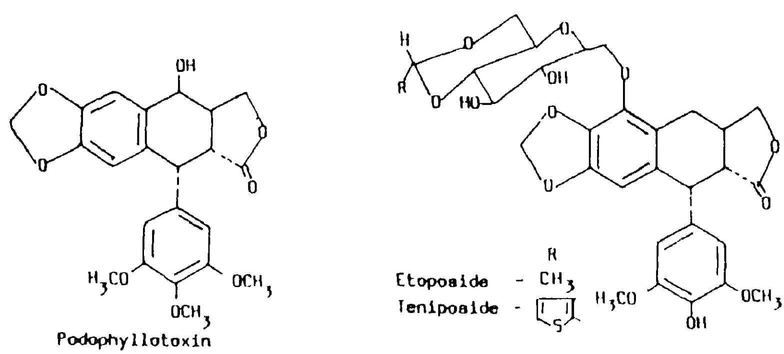
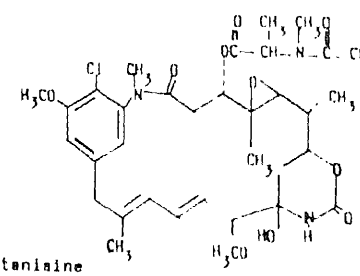


Fig. 20

used by pharmaceutical industry for structural modification producing therapeutically useful anticancer drugs like Podophyllinic acid ethylhydrazide(SP-1) and Podophyllotoxin benzylidene-5-D-glucopyranoside(SP-G). Some cyclic acetals and ketals of 4′-demethyl-epi-podophyllotoxin-beta-D-glucopyranoside show promising activity both *in vivo* and *in vitro*. Two of them, the ethylidene derivatives (Etoposide) and the thenylidene derivative(Teniposide) are marketed as anticancer drugs by Sandoz. In contrast to Podophyllotoxin(a classical spindle poison or antimitotic causing arrest of cells in the metaphase) Etoposide and Teniposide do not affect microtubule assembly. Instead they prevent cells from entering mitosis and arrest cells in the late S or G2 phases of the cell cycle. Epipodophyllotoxin derivatives interact with topoisomerase II[282]. Etoposide is proved to be useful in the treatment of patients with small-cell lung cancer, testicular cancer, Kaposi's sarcoma, lymphoma and leukaemia, while Teniposide in cases of acute lymphatic leukaemia, neuroblastoma and non-Hodgkin's lymphoma and brain tumour in children[283].

Maytansinoids

Genus Maytanus is a source of potential antitumour agents Maytansinoids. Maytanisine, an Ansamacrolide is an antitumour phytoconstituent of exceptional promise obtained from species like Maytanus serrata, *M. emarginata* (Celastraceae)



Maytanisine

Fig. 21

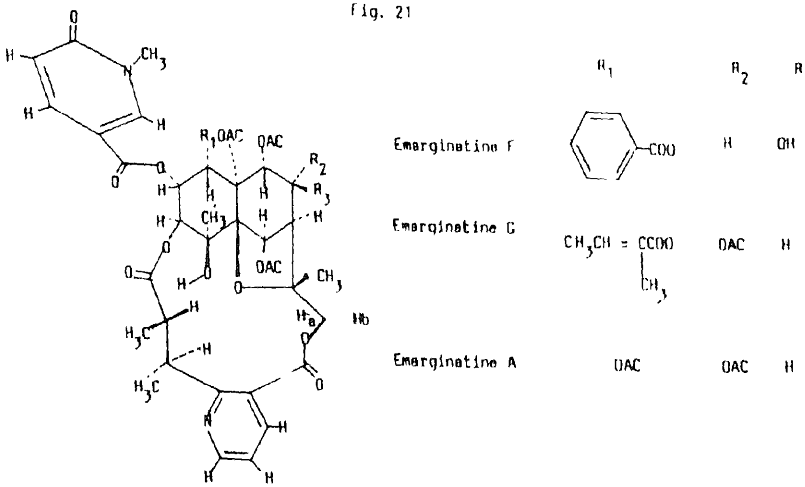


Fig. 21A

and plants like *Putterlickia verucossa* of the same family. It acts at a very low dose through inhibition of mitosis against several experimental neoplasms and has shown a favourable therapeutic index. The highest content (8-9mg/kg) of Maytanisine is reported in *Putterlickia verucossa.* Even though clinical reports of Maytanisine is not much promising, hope for getting good results with its synthetic and semisynthetic derivatives cannot be ruled out. Emarginatine A, F and G and Hippocratine isolated from *M.emarginata*[284] show strong cytotoxicity, specifically Emarginatine F exhibits significant cytotoxicity against human epidermoid carcinoma of neopharynx, ileocical adrenocarcinoma, melanoma, mediloblastoma tumour cells and against murine lukaemia. Emarginatine B shows potent cytotoxicity against human KB cells.

Ellipticine

Ellipticine and 9-methoxy ellipticine, obtained from *Ochrosia elliptica* (Apocynaceae) and other plants of the same family, are Pyridocarbazole alkaloids acting as potent oncological drugs. Ellipticine molecule intercalate between the pairs of DNA and cause a partial unwinding of helical array[285]. Ellipticin gets oxidised *in vivo* mainly to 9-OH-ellipticine, having higher activity. Ellipticine and it's derivatives are quarternized to get water soluble and highly active compounds. 9-OH-elipticine is quarternized to yield 9-OH-2-N-methyl-ellipticinium acetate, a highly active compound, in some forms of breast cancer and also in renal cancer. Moreover lipophilic derivatives viz. 2-butyl-7,10,12-trimethyl-(6H)-oxazolo(4,5-g}pyrido{4,3-b]carbazole(BOPC), 2-(5'-amino pentyl}-7,10,12-trimethyl-(6H)- oxazolo[4,5-g] pyrido[4,3-b]-carbazole(PAPOC) exert antitumour activity by binding externally to the DNA molecule[286]

R = H Ellipticine

R = OCH_3 9-Methoxyellipticine

R = $(CH_2)_3$-CH_3 BOPC

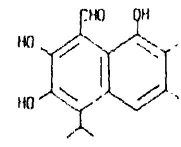
R = $(CH_2)_4$-CH_2-$\overset{+}{N}H_3$ PAOPC

Fig. 22

Gossypol

Gossypol, a Dimeric sesquiterpene found in cotton tribe (Gossypiaceae) occurs usually as dextrorotatory Gossypol with varying optical purity but an enantiomer (-) Gossypol possessing irreversible antifertility activity in males is detected in excess only in one plant *Gossypium barbadense*[287]. Gossypol inhibits human

sperm, has recently been renowned due to the discovery of it's selective toxicity towards cancer cells[288,289,290].

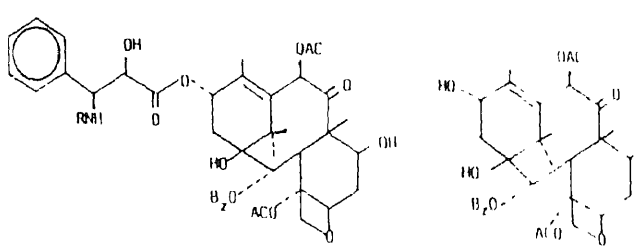


Gossypol

Fig. 23

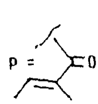
Taxol

Taxol is a novel Diterpenoid isolated from the stem bark (0.01%) of the Pacific yew *Taxus brevifolia*[291] (Taxaceae) and other Taxus species like *T.baccata*, *T.cuspidata*, *T.media*, etc. [292,293,294]. It is established as a clinically acitve anticancer agent showing excellent activity against ovarian and breast cancer[296,297]. It also shows responses in patients with other forms of advanced malignancy including lung cancer, cancer of the head and neck region, malignancy including lung cancer of the head and neck region, malignant melanoma and lymphomas[298]. It shows anticancer activity even in patients resistant to cisplatin. It blocks the cell replication in HeLa cells and fibroblast cells by promoting the assembly of microtubules from tubulin and the microtubules so formed are abnormal and stable to depolymerisation[299]. The exact mechanism by which taxol promates the assembly of tubulin into microtubules is only partially understood. Taxol binds to assembly of microtubules reversibly and approximately one molecule of Taxol binds per tubulin dimer[300].



Baccatin III

R = Bz Taxol



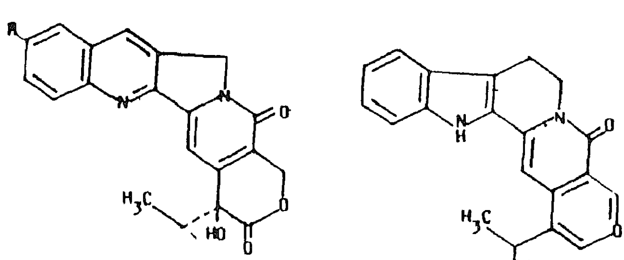
R p = Cephalomannine

Fig. 24

It is insoluble in aqueous medium and so complex emulsion formulation as well as relatively high dose of Taxol is required compared to other antitumour natural products like Vinca alkaloids[301]. The demonstration of clinical antineoplastic activity has led to enormous demand for Taxol and so to encounter the supply as well as the formulation problems, several alternative sources are being investigated. A significant progress has been made for a total synthesis of Taxol but due to the complexity of molecule it is not yet fully achieved[302,303]. Tissue culture technique is a potential source of Taxol specifically cell suspension cultures from high yielding plants are good source of Taxol and related Taxanes(Cephalomanniine, Baccatin III, and 10-deacetyl baccatin)[304]. Needles and twigs of Taxus species are reported to yield a good amount of Taxol and related Taxanes[305,306,307]. Semisynthetic methods for conversion of Taxanes into Taxol are developed[308,309,310] and an Italian company Indena S.P.A has developed a procedure for the isolation of Taxanes on industrial scale. Number of modified Taxol[311] analogues are reported e.g., a side chain analogue RP 56976 has reportedly greater chemotherapeutic potential than Taxol itself. 7-epitaxol shows tubulin polymerisation[312] and consequent inhibition of mitosis. Taxol C shows potent and selective cytotoxicity in the NCI human cell line screen[313].

Camptothecins

Camptothecins are Pyridoindole alkaloids isolated from seeds of *Camptotheca acuminata* (Nyssaceae) and from an Indian plant *Mappia foetida*. Camptothecin is a well known antineoplastic agent showing broad spectrum activity and has shown good response in clinical trials. It is known to have specific activity in topoisomerase I system. Its derivatives like 18-OH-camptothecin[314], 11-OHcamptothecin[315], 10-OHcamptothecin show strong anti-leukemic activity. Besides Angustine, 19-O-methylangustoline[316], 22-OH-acuminatine[317] and 19-OH-mappicine also exert profound cytotoxicity. Camptothecins are reported to cause neurotoxicity and occasionally bone marrow depression and so synthetic analogues of Camptothecin possessing higher antitumour activity and lower toxicity than Camptothecin have



Camptothecin R = H
10-OH-Camptothecin R = OH

Angustoline R = OH
19-O-methyl Angustoline R = OCH_3

Fig. 25

been developed. Two relatively new camptothecin derivatives, CPT-11 and Topotecan show good response in clinical trials. Moreover 9-amino-camptothecin is under clinical trials and the results indicate that the demand for Campothecin is going to increase in the near future [318,319,320]

Bryostanins

Bryostanins are antineoplastic Macrocyclic lactones, obtained from marine *Bryozoa Bugula neritina*. Bryostanins are found to activate protein kinase C without possessing tumour promoting potential[321] and stimulate cellular immune parameters at about same concentrations as used for the generation of direct antineoplastic activity[322]. They also exhibit immunostimulating activities at low concentration and exert immunosuppressive effects in higher doses[323]. Bryostanins 1,2 and 5 are shown to activate polymorphonuclear and mononuclear cells[324]

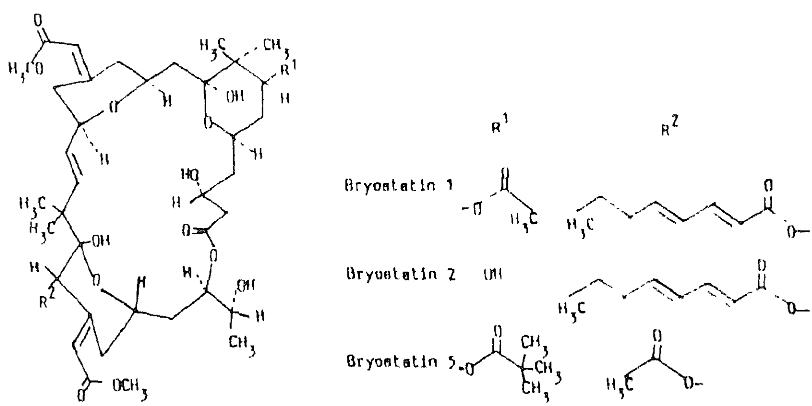
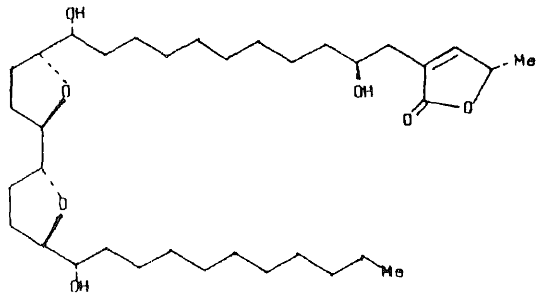


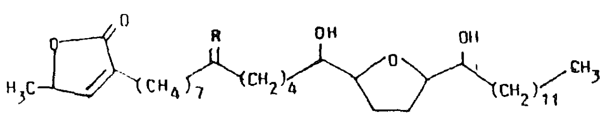
Fig. 26

Acetogenins

Number of highly bioactive Acetogenins are isolateld from Annona species (Annonaceae)[325]. Acetogenins viz. Bullatacin and Bullatacinone isolated from *Annona squamosa* stem bark show significant cytotoxicity *in vivo* against L-1210 murine leukemia in the potent dose range of 50-400ug/kg., being 40-300 times more potent than Taxol[326]. Corossolone and Corossolin isolated from *A muricata* exhibit significant cytotoxicity[327]. The leaves of *A.reticulata* yield Acetogenins like Annoreticuin, Isoannoreticuin, Squamone, Solamin, Annomonicin, Rollinia-statin-2one and Annoreticuin-9-one. These compounds have shown significant cytotoxicity and are currently under evaluation as potential anti-cancer agents[328].



Bullatacin



R = O Corossolone

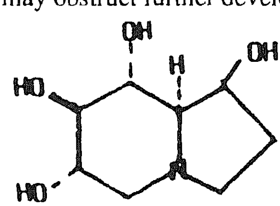
R = OH, H Corossoline

Fig. 27

Anti-aids Agents

Castanospermine

Castanospermine, is a Tetrahydroxyindolizidine alkaloid found in the seeds of *Castanospermum australe* (Leguminosae)[329] and Alexa species. The compound is potent inhibitor of beta-glucosidase, Beta-glucocerebrosinase and lysosom alpha- and beta- glucosidases[330]. Recently it is shown to inhibit replication of HIV[331]. The compound inhibits the enzymes alpha-glucosidases I and II which are important for production of glycoproteins contained within the envelope of the virus[332]. Without the proper glycoprotein coating, the HIV virus is unable to spread to uninfested cells. Several O-acyl derivatives of Castanospermine are 20 times more active than Castanospermine in inhibiting the HIV-virus replication[333]. Higher toxicity of this compound may obstruct further development.



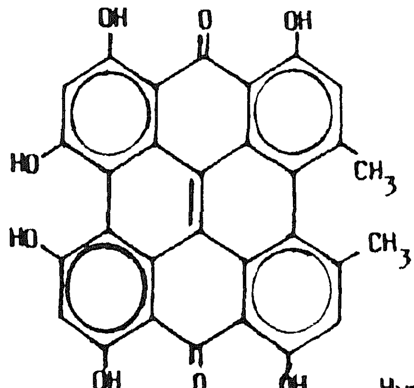
Castanospermine

Fig. 28

Hypericin

Hypericin[334], an aromatic polycyclic dione(an anthraquinone derivative) occurs in certain species of *Hypericum* (Hyperiaceae) such as *H.perforatum*. It shows antiretroviral activity and inhibits the propagation of Friend Leukemia Virus (FLV) and Radiation Leukemia Virus both *in vitro* and *in vivo*. Results of clinical trials indicate Hypericin as a promising drug[335]. Amongst the strains of *H.perforatum* investigated for Hypericin and Pseudohypericin content, Strain R produces highest Hypericin and Pseudohypericin[336,337]. Strain H produces only small amounts of Hypericin and Pseudohypericin.

Tissue culture techniques have been developed to produce Hypericin from *H.perforatum*[338,339].

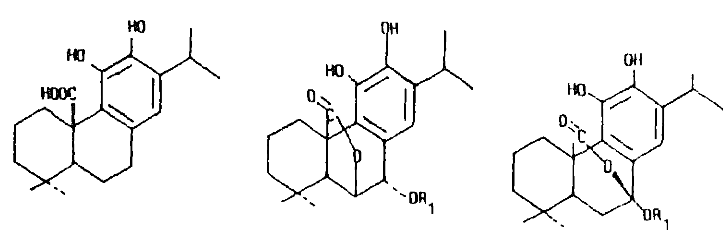


Hypericin

Fig. 29

Cornosolic Acid

Cornosolic acid, a Diterpene isolated from *Rosamarinus officinalis* (Lamiaceae) shows a strong inhibition of HIV-1-Protease activity[340]. It shows cytotoxicity at the dose which is close to effective antiviral dose which suggests that cornosolic acid derivatives with selective antiviral activity can be developed.



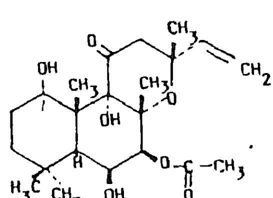
Carnosolic acid Rosmanol Carnosol

Fig. 30

Rosmanol and semisynthetic derivatives 7-O-methyl rosmanol, 7-O-ethyl rosmanol and 11,12-O,O-dimethyl carnasol show greater antiviral activity.

Forskolin

Forskolin, colenol, a Labdane diterpenoid is developed by Hoechst (India) and CDRI Lucknow from the roots of *Coleus forskohlii* (Lamiaceae)[341]. It is reputed for it's hypotensive and cardioactive properties. It stimulates adenylate cyclase, leading to an increase in cellular CAMP[342,343] and thereby shows positive ionotropic effect on heart, lowers blood and intra-occular pressure, reduces inflammation and inhibits platelet aggregation[344, 345]. It exerts inhibitory effect on number of membrane transport proteins and channel proteins[346]. It is reported to have a potential as antimetastatic agent[347]. Clinical studies have focussed on it's cardiovascular and bronchospasmolytic action and on the treatment of glaucoma. Seeing the clinical potential and *Coleus forskohlii* being the sole source for the production of Forskolin, alternatives for the search of Forskolin are being investigated. Although synthesis of Forskolin is reported, it's economic preparation is not achieved. In suspension cultured *C. forskohlii* cells, Forskolin production has only been achieved with morphogenic root forming clumps[348,349]. These root clumps produce a metabolite pattern similar to that of *C. forskohlii* root and hence a biotechnological process using fermentor cultured coleus clumps may be substituted for the field grown plants. Considerable amounts of Forskolin can be obtained using inactive Forskolin-related plant metabolites such as 1,9-dideoxyforskolin through microbial transformation[349]. Recently number of such cell-culture methods are developed using shake flask bioreactors[350,351,352] giving high yields of Forskolin. Synthetic hydrophilic derivatives like 7-beta-D-glycerylforskolin and 7-dimethy-lacrylforskolin are shown to display the same potency of activating platelet adenylate cyclase as Forskolin[353].



Forskolin

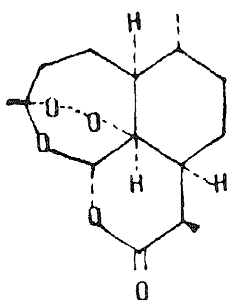
Fig. 31

Artemisinin (Quinghaosu)

Artemisinin is the most promising clinical anti-malarial drug isolated by Chinese scientists from *Artemisia annua*(Asteraceae) in 1971 and highest quantity is found before flowering[354]. Quinghaosu, a herbal extract is used in chinese medicine as a remedy for common cold chills and fever since ancient days[355]. In India Artemisinin (0.1%) is reported to be isolated from *A.annua* growing in Lucknow.

Artemisinin is a sesquiterpene lactone having an endoperoxide moiety essential for it's activity. It shows profound activity against chloroquine resistant *Plasmodium falciparum*[356]. In plants it co-occurs with Artimisitene, Arteannuin B and Artemisinic acid.

The drug affects the integrity of parasite's membrane[357], the mode of action being different from the currently synthetic antimalarials. Recently it has been shown that methoxylated flavonoids like Casticin, Chryolentin found in the plant and Artemesin found in cell cultures enhance the antimalarial activity of Artemisinin[358]. Highly lipophilic nature, comparative non-abundance of this species (Artemisia) and low content of Artemisinin has led to urgent search for alternatives



Artemisinin

Fig. 32

like isolation of other active metabolites from *A annua*; from the plants[359] and by tissue culture technique[360,361,362,363,364,365]; as well as synthesis of Artemisinin and it's analogue Recently it has been reported that methanolic extract obtained from callus of *A.annua* not containing Artemisinin exhibits antiplasmodial activity indicating presence of few unknown compounds[366]. Arteannuin B and Artemisinic acid can be converted to Artemisinin using cell free extracts[367,368] Besides number of recent approaches has led to improved isolation process for Artemisinin from *A.annua*[369,370,371]. Besides this synthetic derivatives like Artemether, Arteether, Sodium artesunate, Artenilic acid and Beta-arteether possessing more potent activity have been developed from Dihydroartemisinin[356]. Sodium artesunate acts rapidly in restoring the consciousness of comatose patients with cerebral malaria Number of synthetic processes are reported[372,373,374] and recently NCL, Pune has developed a short and practical synthesis of Artemisinin[375]

Echinacea species

Roots of Echinaceae species like *E.purpurea, E.angustifolia, E.pallida* (Asteraceae) are described as immunomodulatory drugs. Root extracts are widely used for the unspecific enhancement of immune system[376]. Alkamide fraction isolated from

E.angustifolia is shown to possess immunostimulant activity[377,378]. Recent investigations have shown that primary metabolites like Arabinogalactans and Arabinogalactan proteins of the species are responsible for immunomodulating and antiviral activity[379,380, 381,382,383,384], an acidic Arabinogalactan obtained from cell cultures of *E.purpurea*[385,386] being the most active one[387]. Besides Heteropolysaccharides, presence of immunomodulatory active Proteins with different Amino acid compositions are reported in the root extracts of Echinaceae species[388].

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Dioscorea deltoidea Hook. Distribution and Agrotechnology

A.K.Rishi, M.K.Bhan & P.L.Dhàr

Regional Research Laboratory
Jammu

Introduction

Plants of genus *Dioscorea* (Family *Dioscoreaceae*) are perennial climbers with tuberous roots. Approximately 600 species of *Dioscorea* occur rather abundantly in tropical area, sub tropical regions with a few species in the temperate regions[1]. Only 15 species are reported to contain steroidal sapongenin chiefly diosgenin. Most of the world production of diosgenin is met with central American species *D.floribunda* and *D.composita* both growing wild in central America[2]. In India *D.deltoidea* and *D.prazeri* occuring wild in North West and North East Himalayas respectively are the natural sources of diosgerin. *Dioscorea deltoidea* is found in India, Pakistan, Nepal and Bhutan extending to South Western China while *D. prazeri* occurs in Eastern India, Burma and northern parts of Malaysia[2]. Mexico, Gautmala, Costa Rica, India, China, and some countries in Europe and Africa are the major *Dioscorea* producing countries[3].

Diosgenin is the major raw material used in the synthesis of corticosteroids, sex harmones and antifertility compounds[4]. Out of the steroid drug precursors diosgenin accounts for 50% of the total steroidal products of the World[2]. Present requirment of steroidal drugs in terms of Diosgenin in the world is approximately 3000 tonnes. The total annual requirement of diosgenin by the pharmaceutical industry of our Country is 150 tonnes, while the total production of diosgenin in India is about 30 tonnes annually[5]. The rest of the requirement is met through imports in the form of diosgenin and drug intermediates. The total estimated

demand of corticosteroids is 9253 kg, whereas the actual production is 3941 kg[6]. The synthesis of diosgenin not being commercially feasible[7] for production of steroidal drugs, makes it necessary, rather obligatory, for the pharmaceutical industry to resort to natural source for the procurement of this raw material. While *D.prazeri* yields impure diosgenin, it has been reported that diosgenin percentage of *D.deltoidea* varies from 0.6 to 10.3[8]. Hence, *D.deltoidea* seems to be the best natural source of diosgenin.

Cultivation of *Dioscorea* in India became necessary in view of the growing demand of the pharmaceutical industry coupled with the indiscriminate extraction of *D.deltoidea* from the forests which resulted in fast depletion of this species so much so that it has been declared a threatened species. Attempts to domesticate *D.deltoidea* in India were started during fifties, but only partial or little success was obtained[10]. Attempts by a number of pharmaceutical concerns to cultivate this species was not encouraging[12]. After finding it difficult to grow *D.deltoidea* on commercialy viable basis, *D.composita* and *D.floribunda* were imported from central America. A collaborative programme was launched by ICAR, CSIR and Ford Foundation during 1967 to 1969 to explore the possibility of domesticating this species by vegetative propagation in Srinagar, Delhi, Bangalore and Lucknow. The studies showed that *Dioscorea deltoidea* contained the highest amount of pure diosgenin, but was too slow growing for adoption as a commercial crop[10,13]. Most of their work has been reported in the field of propagation, cultivation and agronomy of *Dioscorea deltoidea*. The present paper highlights the detailed agro-technological studies carried out under Kashmir conditions and also its distribution pattern and availability in North-West Himalayas.

Distribution

Dioscorea deltoidea grows wild throughout North Western Himalayas and extends from Kashmir, Himachal Pradesh, Darjeeling to Sikkim, Nepal and Bhutan at an altitude ranging from 1000-3500 metres. In J&K it is widely distributed in the forests of Bhadarwah, Kishtwar and Doda (Jammu) and forests of Ovra (Pahalgam), Shikargah, Kangan and Pirpanchal in Kashmir. In Himachal Pradesh it is widely distributed in Catchment areas of the rivers of Ravi and Beas in Chamba and Kulu Valley. It is fairly common in upland regions of Kangra valley and Kinnaur. In Uttar Pradesh, it is available in upper reaches of Tons and Jamuna valley,dry temperate regions of Uttar Kashi and Chamoli in Garhwal and in Kumaon Hills. In Sikkim the plant occurs in Chughthang Singhik regions of Sikkim. Detailed survey by Abrol et al[14] in the mountainous regions of J&K, H.P., and Punjab has shown that *D.deltoidea* grows better in areas where annual raintall varies between 100 to 200 cm and minimum temperature in winter goes below freezing point and the maximum temperature in summer does not go up to 32°C. It has also been observed that *Dioscorea deltoidea* grows generally on the northern and eastern slopes in shady but well drained conditions. The tubers of Poonch area of J&K and H.P

contain poor quality of diosgenin content whereas the tubers from Pahalgam, Bhadarwah, Katra, Batote and Kalu contain about 4% diosgenin. The annual availability/out turn of *Dioscorea deltoidea* collected from various forests of H.P. and J&K is given in Tables 1-3.

Collection of Rhizomes

The time of collection of rhizomes is important since its diosgenin content varies from season to season. The rhizome starts sprouting in the month of May and vines

Table 1 — Annual availability of *Dioscorea deltoidea* from various forest divisions of HP.

Forest Division	Quantity (q)
Kinnaur	800
Kulu	700
Seraj	600
Rohru	250
Mandi	160
Nachan	200
Kolgash	250
Kanjora	180
Dalhonsa	150
Chamba	600
Pangi	1000

Source: Sarin & Chopra 1985

Table 2 — Average out turn of *Dioscorea deltoidea* from the forest division of HP.

Forest area	Quantity(q)
Kinnar	853
Kulu	1390
Seroj	200
Rohru	156
Nachan	200
Kotgara	1129
Dalhonsic	125
Chamba	200
Simla	26

Source: Sarin & Chopra, 1985.

Table 3 — The annual out turn of dried *Dioscorea deltoidea* rhizomes from the forests of J&K from 1980-1994.

Year	Quantity (q)
1980-81	498.45
1981-82	1185.74
1982-83	200.00
1983-84	160.90
1984-85	196.00
1985-86	488.00
1986-87	450.00
1987-88	496.00
1988-89	248.00
1989-90	700.00
1990-91	700.00
1991-92	630.00
1992-93	487.50
1993-94	368.00

Source: Department of J&K forest Statistical Cell.

bear flowers in September and the seeds are ripe by November-December[16] Abrol *et al.*, 1963 observed that the best time of collection of rhizomes is in the months of January to March when the plants are dormant. It is not advisable to collect the rhizomes in December because the fields are covered with snow. The percentage of diosgenin was reported to vary from 3.8 to 4.6 at the time of sprouting (May), 3.8 at the folwering stage (September), 4.0 during fruiting stage (November), 4.1/4.2 at seed set (December) and 4.6 dormancy (January-March)

Agrotechnology of *Dioscorea deltoidea*

Climate

It is a temperate plant and requires an annual rainfall varying between 100 to 200 cm. Minimum temperature below freezing point during winter helps in release of gibberellins and, thereby, breaking dormancy of the tubers. Even when Srinagar maximum temperature goes upto 34°C we have observed good growth of *D.deltoidea*. Frequent irrigation can avert the transpiration losses during summer and in case Sprinkler irrigation is practised, wilting of the leaves can be avoided.

Soil

A well drained loose textured soil rich in humus with a *p*H 6.8 to 7.2 results in healthy growth of the tubers.

Propagation

Dioscorea deltoidea can be propagated from seed, leaf node cutting or from rhizomes cuttings.

From Seeds: According to Martin and Gaskin[17] propagation from seed is considered the most reliable method under Peurto Recan condition though asexual propagation may be used profitably. Under certain conditions Khakazi *et. al.*[18], propagated *Dioscorea deltoidea* by sowing seed in nursery and the two years old plants were transplanted in the field. After 4 years of growth the fresh weight of rhizomes/plant was 216 gm. Our observations did not show encouraging results, as the seed propagation trial in a growing season gave an average 12 gm of root mass which was highly disappointing. In India, however, propagation is mostly done vegetatively.

From Rhizomes: The plant can be easily propagated from rhizomes. Tubers (75 gm) planted and harvested after 32 months gave optimum tuber yield of 20.79 q/ha with 2.08 percent of diosgenin[19]. It was observed at Peshawar that tuber pieces with 5 cm diameter having one or two buds gave higher total number of plants Khan *et.al.*[20], and Bhat and Bindroo[21], reported that dormancy in tubers can be broken by chilling tubers at 2°C to 0.5°C for 60 days resulting in 95% sprouting and such chilling could save time at the start of fresh cultivation. (Abrol *et. al.*, 1963)[16] observed that rhizome pieces 3 cm in length and having one bud are quite suitable for raising plantation and early spring is the optimum time for planting the cuttings where 100% sprouting is expected. Our observation on 1162 plants drawn out from the field randomly indicated a positive correlation of r = 0.67 between bud number and tuber weight. Based on this finding we planted rhizome cuttings having 3 to 4 buds weighing around 50 gms in the month of November and observed maximum yield of rhizomes after 3 years of growth (Fig 1).

Cultivation

Land Preparation

Land should be prepared thoroughly 4-5 times with a mould board plough. It should be thoroughly mixed with farm yard manure or forest humus. 14 tonnes/ha of FYM should be applied at the time of field preparation.

Pretreatment of Tubers before Plantation

The tubers are susceptable to a number of soil borne diseases, unless treated with proper fungicide. After trying different fungicides it was found that tubers dipped in 0.2% of brassicol for 10 minutes before plantation gave better results.

Method of Planting of Tubers

Planting of tubers should be done in furrows. Deep furrows are made at 60 cm distance with the help of plough. The tuber pieces are planted at a distance of 30 cm about 5 cm below the soil level. The new sprouts need immediate staking. After

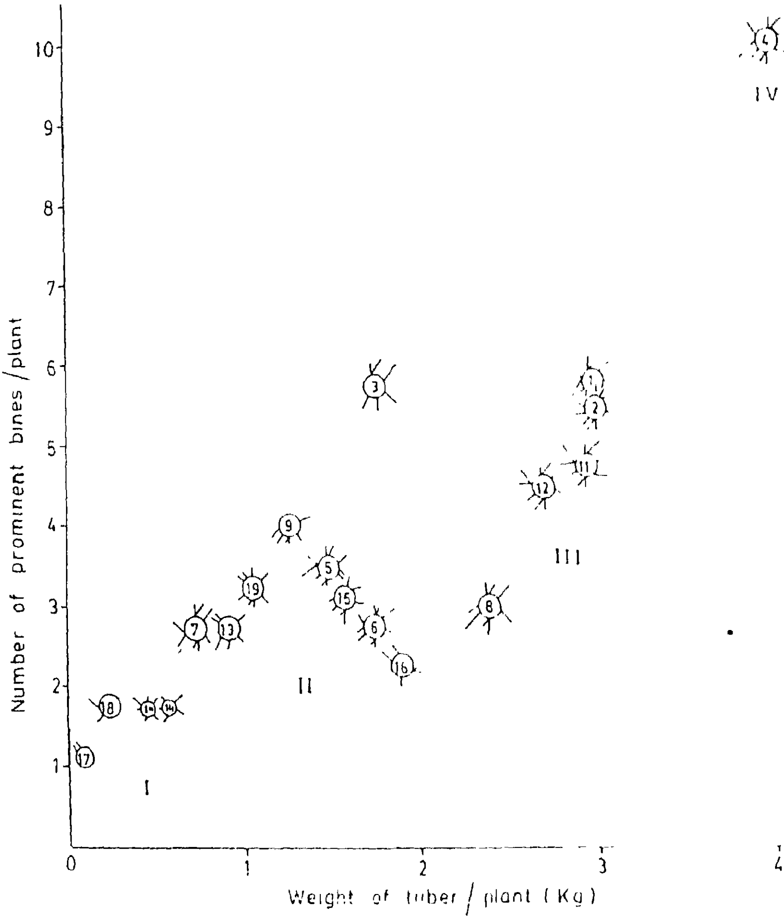


Fig.1 SCATTER DIAGRAM IN METROGLYPH ANALYSIS

one month the plants are earthened up, thus making ridges in between the row into furrows and converting the original furrows into ridges with plants on them. Spacing between plant to plant and row to row should be 30cm ×60cm respectively.

Supporting System

Dioscorea vines being essentially climbers need support for their maximum growth. An experiment in RBD was laid out in Regional Research Laboratory (Srinagar) experimental field on a clayey loam soil the structure of which was modified by a dressing of 14 tonnes/ha of FYM on an area of 0.10 ha. The treatment consisted of two different heights *viz.* 1.8m and 5.4m of trellis and no support. Rhizome cuttings having 3 to 4 buds and weighing around 50 gms were planted on

ridges keeping plant to plant and row to row distance of 30cm × 60 cm. A uniform dose of NPK (50, 100, 75 kg/ha) were applied in equal splits in middle of April and July for three years. After three years of growth trellis height of 5.4m resulted in an yield of 13.04 tonnes/ha of *Diosocrea* rhizomes compared to 8.72 tonnes and 6.63 tonnes/ha with 1.8 m trellis height and no support. Thus a trellis height of 5.4 m is essential for the good growth of *Dioscorea deltoidea.*

Cost of Trellis

The system tried at Regional Research Laboratory, Srinagar consisted of a 5.4 m trellis height errected with the help of G.I. Pipes (20 ft long with 2" dia.) spaced at 10m×20m apart in the field. Stranded wires are used on the four sides of the field. Interconnecting G.I. wires which come on top of two rows were of 8 SWG. Each plant was supported by nylon string and tied to the overhead wire. The complete cost of trellis based on angle iron structure and the cost of cultivation is given in Table 4

Weeding

Initially the vines are weak and tender. They can not compete efficiently with the sorrounding weeds. Weeding is essential for the first year as and when required In remaining two years the weeds are reduced due to shade provided by the prolific growth of *Diosocrea* plant with the result that the plants can compete with the weeds, more successfully, thereby, reducing weeding operations.

Irrigation

Under Kashmir conditions *Diosocrea deltoidea* does not need much irrigation. However, during months of June to August, it should be irrigated once every fortnight. The newly planted crop of *Dioscorea deltoidea* has to be irrigated when needed during the first year.

Fertilizer Requirement

Dioscorea species responds well to organic manure and nitrogenous fertilizers. In case of *Dioscorea deltoidea*, 14 tonnes of FYM/ha is recommended before plantation. Fertilizer trial with 135 kg each of N&P/ha in Kagan Valley of Pakistan gave poor yield of 1.27 tonnes/ha rhizomes[22]. A field experiment in RBD was laid during 1981-84 with six levels of Nitrogen (0,40,80,120,160,200 kg/ha) to investigate the optimum dose of Nitrogen. A basal dose of 100 kg P_2O_5 and K_2O each were applied at plantation time. Nitrogen was applied in the form of urea in two equals plits in mid-April and mid-June. The tubers were harvested in November, '84. The investigation revealed that nitrogen fertilization up to a particular level increased tuber yield/ha and marginally enhanced diosgenin percentage in the tubers of three year

Table 4 — The cost of Cultivation of Selected clones of *Dioscorea deltoidea*/Economics of Cultivation/ha Harvest after every 3 years

	Duration of the crops 3 years		
A.	*Capital cost of Trellis work (once only)*		
		Quantity	*Amount* (Rs.)
	1. Angle iron fixture 18'×2"×2"	(70 Nos)	20,790
	2. G I. wire 8 SWG	15 q.	27,000
	3. Fixation of wire work & Stranded wire etc.		10,000
		Total	57,790
B.	*Recurring expenditure*		
	Items	*Expenditure in Rupees*	
	1. Land Preparation		7,000
	2. Planting material & plantation		28,000
	3. Purchase of Fertilizer, manure and its application		20,000
	4. Weeding & hoeing		15,000
	5 Irrigation		6,000
	6. String (Nylon Yarn)		9,000
	7 Stringing charges		5,000
	8. Harvesting Cleaning & Post drying of tubers		20,000
	9 Miscellaneous		6,000
	10 Land Rent		24,000
		Total	1,40,000
C.	*Returns after three years*		
	i) Yield of tuber/ha		23.0 tons
	ii) Gross returns/ha at the rate of Rs. 10/kg		2,30,000
	Profit		
	Net returns in three years		90,000
	Net return per year/ha		30,000

crop. The diosgenin percentage showed a decrease above the applied level of 80 kg/ha although the yield, length and girth of tubers along with the yield of diosgenin showed continuous increase (Tables 5 & 6). The optimum economic dose of nitrogen from the quadratic equation worked out 95.12 kg/ha with the estimated tuber yield of 16 tonnes/ha[23].

Diosocrea floribunda and *D. composita* respond well to nitrogen (300 kg/ha) application[24]. Keeping in view the above facts a fertilizer trial of NPK was carried

Table 5 — Effect of Nitrogen levels on the tuber yield, length, girth, diosgein percentage and diosgenin yield of tubers in *Dioscorea deltoidea*

Level of Nitrogen	Tuber yield/ ha (tonnes)	Tuber length/ plant (cm)	Tuber girth/ plant (cm)	Diosgein %	Diosgenin yield kg/ha
0	10.114	14.30	29.20	3.50	353
40	13.834	16.40	33.10	3.75	510
80	15.892	18.20	36.50	4.32	686
120	16.140	19.10	38.30	3.87	625
160	17.080	19.30	38.70	3.76	641
200	17.429	19.30	38.90	3.70	644
LSD 5%	0.080	1.737	1.07	0.34	55

Table 6 — Effect of N, P, K on the growth of *Dioscorea deltoidea*

Treatment	Tuber yield/ plant (kg)	Tuber length/ plant (cm)	Tuber girth/ plant (cm)	Diosgenin (%)
N_1	0.4215	14.40	34.72	3.75
N_2	0.4132	14.20	33.78	3.70
N_3	0.4461	15.10	36.69	3.61
CD at 5%	N.S	N.S.	N.S.	0.07
P_1	0.4162	14.53	34.50	3.71
P_2	0.4224	14.70	34.82	3.73
P_3	0.4221	14.68	34.67	3.72
CD at 5%	N.S.	0.09	0.12	0 03
K_1	0.4113	13.97	32.97	3.70
K_2	0.4238	14.50	34.20	3.68
K_3	0.4457	14.82	35.76	3.67
CD at 5%	N.S.	N.S.	N.S.	0.02

out during 1986-89 at RRL, Experimental field, Srinagar. The experiment was carried out on a loamy soil in a split plot design with three levels of Potash 75(K_1), 100(K_2) and 125(K_3) kg/ha in main plot, three levels of Phosphorus 100(P_1), 150(P_2) and 200(P_3) kg/ha in sub plot and three levels of nitrogen 150(N_1), 200(N_2) and 250(N_3) kg/ha in sub plot respectively replicated four times. After three years

of growth the data was recorded on tuber yield/plant, tuber length/plant and tuber girth/plant and analysed statistically.

The results indicated that neither main treatment, nor the sub treatment nor the sub-sub treatment recorded any significant effect on the tuber yield/plant However, all the treatments were found to be significant for diosgenin percentage (Table 6) The interaction K×N, N×P, K×N×P were significant and the maximum tuber yield (23.5 tonnes) and diosgenin yield (8.81 q) was obtained with low level of nitrogen and high level of potash and phorphorus viz. 125(K_3) 200(P_3) 150 (N_1) kg/ha and is recorded in Table 7[25]. Thus *Dioscorea deltoidea* does not need high doses of nitrogen/ha as has been observed in *Dioscorea composita* and *Dioscorea floribunda*.

Genetic Variability

Bammi *et al.* [10] reported a range of variability in diosgenin content, 0.8 to 10.5%, on dry weight basis in clones of *D. deltoidea* collected from North Western Himalayas. Bhardwaj *et. al* [24] reported high heritability for the diosgenin content and vine length in *D. deltoidea* collected from H P Bindroo & Bhat 1986[27] observed a great variation in number of characters in natural population of *D.deltoidea*. Bindroo & Bhat 1986[28] studied statistically parameters estimates for some traits of *Dioscorea deltoidea*. Number of growing heads on tuber was fairly a suitable trait, while traits like number of aerial shoots arising from the tuber and number of leaf veins were not suitable and were greatly influenced by the environment. Bharadwaj *et al.*[26] studied the genetic aspect of clones of *Dioscorea deltoidea* available in four districts of Himachal Pradesh; Clones of Jassorgah, Saloh, Gudiali and Chhutri have the highest amount of diosgenin percentage (3.5, 3.14, 3.01 and 2.5) and also gave high yield of rhizome varying from 59-80 gm/plant after 18 months of growth. The initial weight of rhizome piece planted was 46-50 gm. Collections from Chamba district show the maximum desirable variability for diosgenin content, whereas collection from Kinnaur district gave low yield and low amount of diosgenin. Heritability and genetic advance was high for diosgenin content (159.02 and 54.31), (112.54 and 26.56) and vine length. Diosgenin percentage the most desirable character for selection showed 27.24% genotypic coefficient of variation followed by vine length (17.3%).

Three hundred plants were selected at random by us from the *Dioscorea deltoidea* population of diverse origin collected from different parts of Kashmir and grown under uniform cultural conditions at Laboratory farm. Plants of uniform age (3 years) were taken and observations were recorded on tuber yield/plant, tuber girth/plant, leaf area/plant, number of bines/plant and the weight of vine/plant. Simple, partial and multiple correlation between tuber yield and other characters were taken. All the characters under study were highly variable. The maximum

Table 7 — Effect of different combination of NPK on the growth of *Dioscorea deltoidea*

Treatments	Tuber yield/ plant (kg)	Tuber length/ plant (cm)	Tuber girth/ plant (cm)	Diosgenin (%)	Tuber yield/ ha (q)	Diosgenin yield/ ha (q)
$K_1 P_1 N_1$	0.349	17.01	34.10	3.72	139.6	5.19
$K_1 P_1 N_2$	0.407	18.02	35.20	3.74	162.8	6.09
$K_1 P_1 N_3$	0.509	19.30	38.67	3.82	203.6	7.77
$K_1 P_2 N_1$	0.305	16.57	33.68	3.76	122.0	4.58
$K_1 P_2 N_2$	0.397	17.98	35.00	3.75	158.8	5.91
$K_1 P_2 N_3$	0.473	18.76	37.69	3.78	189.2	7.15
$K_1 P_3 N_1$	0.396	18.00	37.40	3.73	158.4	5.91
$K_1 P_3 N_2$	0.374	17.92	35.00	3.72	149.6	5.56
$K_1 P_3 N_3$	0.489	19.10	38.23	3.79	195.6	7.41
$K_2 P_1 N_1$	0.379	17 61	35.00	3.71	151.6	5.62
$K_2 P_1 N_2$	0.453	18.26	37.67	3.74	181.2	6.68
$K_2 P_1 N_3$	0.390	17.24	35.00	3.68	156.0	5.74
$K_2 P_2 N_1$	0.450	18.21	37.60	3.71	180.0	5.68
$K_2 P_2 N_2$	0.398	17.50	35.60	3.73	159.2	5.94
$K_2 P_2 N_3$	0.523	21.30	41.10	3.87	209.2	8.09
$K_2 P_3 N_1$	0.380	17.23	34.98	3.79	152.0	5.76
$K_2 P_3 N_2$	0.401	17.74	35.00	3.78	160.4	6.06
$K_2 P_3 N_3$	0.437	18.05	35.10	3.77	174.8	6.59
$K_3 P_1 N_1$	0.438	18.01	35.10	3.71	175.2	6.50
$K_3 P_1 N_2$	0.484	19.24	38.23	3.72	193.6	7.20
$K_3 P_1 N_3$	0.335	17.00	34.12	3.71	134.0	4.97
$K_3 P_2 N_1$	0.505	20.70	41.70	3.77	202.0	7.62
$K_3 P_2 N_2$	0.303	17.00	34.62	3.75	121.2	4.54
$K_3 P_2 N_3$	0.447	18.01	35.14	3.77	178.8	6.74
$K_3 P_3 N_1$	0.589	22.01	42.34	3.74	235.0	8.81
$K_3 P_3 N_2$	0.497	19.70	39.67	3.72	198.8	7.39
$K_3 P_3 N_3$	0.400	17.88	37.35	3.78	160.0	6.04
CD at 5%	0.147	2.34	3.67	0.12	34.41	0.78

range of variation, 0.8-4.1 kg with a mean value of 1.69 kg was observed in tuber yield/plant. The number of bines/plant, tuber girth/plant and leaf area/plant varied from 2.25-22.50, 17.80- 88.07 cm and 5.61-21.72 cm^2 with a mean value of 11.63, 47.67 and 13.76 cm^2 respectively. Highly positive and significant correlation was observed between the tuber yield/plant and number of bines/plant, r=0.82 and leaf area/plant, r=0.76 (Table 8). While partial correlation coefficient between tuber yield/plant and leaf area/plant was positively significant when tuber girth, number of bines and weight of vine were kept constant only tuber yield/plant and leaf area/plant were positively significant after eliminating the effect of weight of vine and number of bines. Tuber yield/plant was positively significant with tuber girth/plant when the effect of leaf area, number of bines and weight of vine were eliminated. The tuber yield/plant, however, was not significant when the effect of other variables were eliminated in combination with the number of bines/plant positively significant correlation was observed even when the effect of other variables were removed either singly or in combination (Table 9) Multiple correlation indicates that leaf area/plant, number of bines/plant and weight of vine/plant (r=0.89) were better combination than other characters for enhancing the tuber yield. With the combination of four variables the relative contribution decreases (Table 10) Rishi *et al.*.,[29] studied the extent of variation due to genetic and nongenetic factors, heritability and path analysis in 19 diverse genotypes of *Dioscorea deltoidea*. Phenotypic variability was higher than the genotypic variability for all the characters High heritability was observed for weight of vine/plant, leaf area/plant and tuber yield/plant. Generally genotypic correlation was higher than the phenotypic correlation. The path analysis showed that leaf area/plant and number of vines/plant had the highest direct effect on tuber yield (0.4273, 0.4017) The direct effects of tuber girth/plant and number of buds/plant on tuber yield/plant were negligible. The indirect effects of various characters on tuber yield/plant showed that tuber girth had the highest indirect effect, 0.3386 followed by the number of vines/plant, 0.3033 through the leaf area/plant. The same population was subjected to factor analysis[30]. In factor analysis significant traits were correlation in all possible combinations at the genotypic levels. The weakest correlation was observed between the length of tuber plant and leaf area/plant, r = 0.0452. All the characters studies were correlated with the tuber weight/plant The highest correlations were observed with the number of prominent bines/plant, r = 0.9280 followed by leaf area, r=0.9028 and lowest correlation was observed with length of tuber/plant, r=0.6032. Factor analysis showed the number of prominent bines/plant to be the most important yield component. Direct improvement in this character would improve yield potential without effecting other plant characters.

Table 8 — Simple correlation coefficients in *Dioscorea deltoidea*

Character	Leaf area/ plant	Tube girth/ plant	Number of bines/ plant	Weight of vine/ plant
Tuber yield/plant	0.7664**	0.7684**	0.8246**	0.6370**
Leaf area/plant		0.6793**	0.5898**	0.5832*
Tuber girth/plant			0.7239**	0.4112
Number of bines/plant				0.5434*

* P = 0.05

** P = 0.01

Table 9 — Partial correlation coefficient in *Dioscorea deltoidea*

	2		3		4		5
r12	0.7664**	r13	0.7684**	r14	0.8241**	r15	0.6370**
12.3	0.5205*	13.2	0.5256*	14.2	0.7181**	15.2	0.3643
12.4	0 6130**	13.4	0.4391*	14.3	0.6079**	15.3	0.5504*
12.5	0.6306**	13.5	0.7203**	14.5	0.7394**	15.4	0.3979
12.34	0.3924	13.24	0.2295	14.23	0.6051**	15.23	0.4853*
12.35	0 3636	13.25	0.4809*	14.25	0.6846**	15.24	0.2201
12.45	0.5424*	13.45	0.0452	14.35	0.5111*	15.34	0.4281*
12.345	0.2832	13.245	0.2118	14.235	0.5328*	15.234	0.3691

*P = 0.05; ** P = 0.01

Correlation of yield/plant with : 2, leaf area/plant; 3.tuber girth/plant; 4.Number of bines/plant; 5 weight of vine/plant.

Table 10 — Multiple correlation coefficients in *Dioscorea deltoidea*

		Percent contribution
r1(23)	0.8372**	70.09
r1(24)	0.8944**	79.95
r1(25)	0.8013**	64.20
r1(34)	0.8612**	74.16
r1(35)	0.8454**	71.47
r1(45)	0 8547**	73.05
r1(234)	0.8997**	80.94
r1(235)	0.8673**	75.22
r1(245)	0.8998**	80.90
r1(345)	0.8884**	78.92
r1(2345)	0.8862**	78.53

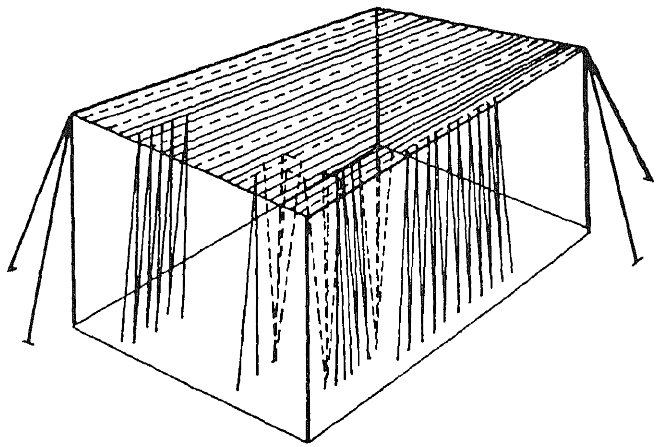
**P = 0.01

Germplasm Evaluation

The meteroglyph analysis was made to study the extent of analysis variation both in quantitative and qualitative characters of the germplasm population of *Dioscorea deltoidea* collected from different parts of J&K.

Ninteen accessions/genotypes of diverse origin collected from different parts of Kashmir were cut into ~50 gm pieces with two buds each and planted on ridges in the experimental field of RRL, Srinagar keeping plant to plant and row to row distance of 30cm × 60cm in a randomised block design with four replications. The plot size of each entry consists of two rows and each row consists of twenty plants. All the agricultural operations were carried out as described earlier[29]. Observations were recorded on fifteen plants from each replicate on number of bines/plant, weight of vine/plant, diameter of the stem, length of the tuber, girth of tuber, number of buds/plant/tuber, number of growing balls, leaf area/plant, tuber yield/plant and number of prominent bines/plant. Diosgenin was estimated spectrophotometrically[31] after three years of growth. There exists a lot of variation in all the characters under study (Table 11). The weight of tuber/plant ranged from 0.065 to 4.00 kg with a mean value of 1.687 kg and the percentage of diosgenin varied from 2.90 to 4.93 with a mean value of 4.046%. The accession 4 gave the highest tuber weight of 4 kg/plant with 4.42% diosgenin while the accessions 5,6,10,12,13,14,15,16,18 gave more than 4% diosgenin with an average weight of tuber viz. 1.35 kg. The girth of the tuber varied from 19.80 to 66.075 cm with a mean value of 47.668 cm whereas the tuber length varied from 10.825 to 43.450 cm with a mean value of 31.434 cm. The accessions 12,13 and 14 have the maximum girth of 66.075, 66.025 and 64.850 cm and the tuber length of 43.225, 32.400 and 42.575cm respectively The number of prominent bines varied from 1.250 to 10 with a mean value of 3.697 whereas the leaf area varied from 5.613 to 21.759 cm^2 with a mean value of 13.759 cm^2. The maximum prominent bines and leaf area was observed in accession 4 and minimum in 17. Metroglyph and index score analysis was carried out according to the method suggested by Anderson 1957[32]. The class interval for various morphological traits and symbols used for different characters is represented in Table 12. Each accession is represented by semi circle, the X-coordinate of each circle being the weight of tuber/plant/accession and the Y-coordinate being the number of prominent bines/plant/accession. The other characters have been represented by rays at different positions on the graph. The scatter diagram shows the following four complexes (Fig. 2).

Complex I: comprises accessions (17,18,10,14). Its main characteristics are few number of bines, least weight of vine, minimum tuber girth, minimum number of growing balls and buds, moderate to high percentage of diosgenin and average length of tuber. The accessions (10,14,18) have more than 4% of diosgenin. The accessions are represented by a minimum of one ray and maximum of five rays.



SHOWS STRINGING PATTERN OF DIOSCOREA DELTOIDEA

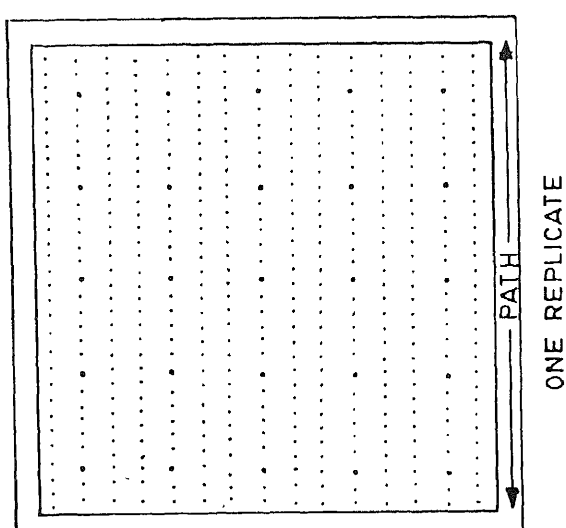
FIGURE-2

SHOWING STRINGING PATTERN OF HOPS

SHOWS STRUCTURE OF ONE BLOCK

SHOWS GUY

SHOWS TRELLIS PATTERN



SCALE 1mm = 5cm ON FIELD

FIGURE-3

.... SHOWS DIOSCOREA PLANTS (ALSO RIDGE APEX)

.....• SHOWS HOP PLANTS

Table 11 — Growth performance of 19 accessions under Kashmir conditions (Three years old plantation)

Locations	I	II	III	IV	V	VI	VII	VIII	IX	X
1. Boniyar	18	5	170.000	1.375	34.950	50.200	56.750	2.975	16.415	3.86
2. Chandigam	19	5	142.500	1.525	43.450	60.150	86.750	2.975	16.310	3.75
3. Sonamarg	16	5	250.000	1.325	34.300	47.650	48.250	1.787	13.842	3.38
4. Bhaderwah	22	10	412.500	1.475	42.575	64.850	99.500	4.00	20.909	4.42
5. Katra	12	3	80.000	1.225	30.500	53.375	69.750	1.612	12.041	4.42
6. Lolab	12	2	182.500	1.325	31.775	45.275	76.250	1.775	13.797	4.66
7. Handwara	9	2	285.000	1.475	28.600	38.200	68.750	0.775	16.663	3.70
.8. Poonch	12	3	162.500	1.325	38.125	54.00	95.750	2.400	14.182	2.99
9. Kistwar	2	1	128.000	0.850	10.825	17.800	14.250	0.065	5.613	3.60
10. Banihal (Upper Munde)	10	3	197.500	1.450	32.400	48.925	67.00	1.500	21.724	4.93
11. Bijhame	12	4	37.500	1.500	38.675	59.125	116.250	2.987	15.284	3.51
12. Shopian	15	4	262.500	1.475	43.225	66.075	145.250	2.687	18.858	4.18
13. Kupwara	9	2	105.000	1.225	32.400	66.025	59.500	0.937	18.285	4.70
14. Verinag	4	1	107.500	1.225	23.500	31.775	53.000	0.537	12.886	4.03
15. TRAL (Shikargah)	10	1	65.000	1.200	24.825	32.100	63.250	0.462	12.223	4.64
16.Aharabal	6	2	85.000	1.375	23.150	43.075	57.500	1.925	6.521	4.44
17.Pahalgam	15	3	57.500	1.200	31.800	50.00	84.500	1.087	7.687	3.87
18.Batote	5	1	130.000	1.075	21.025	29.900	32.250	0.240	5.822	4.32
19.Bandipara	7	4	137.500	1.375	31.150	46.400	96.00	1.275	12.358	3.45
Range	2-22	1-10	57.500-612.500	0.850-1.525	10.825-43.450	17.800-66.075	14.250-145.250	0.065-4.000	5.613-21.724	2.99-4.93
Mean	11	3	182.526	1.310	31.434	47.668	73.184	1.687	13.759	4.046
CD at 5%	4.410	1	62.705	0.1133	5.7932	9.2195	19.035	0.6415	2.3631	N S

I: Bines/plant, II: Prominent bines/plant; III: Vine weight/plant, IV Stem diam. (cm); V Tuber length/plant(cm), VI: Tuber girth/plant(cm), VII. Growing buds/plant; VIII. Tuber weight/plant(kg); IX. Leaf area(cm^2), X: Diosgenin(%)

Complex II: contains accessions (7,12,19,5,15,6,16) and is represented by minimum of 6 rays and maximum of 8 rays. The main features of this complex are average tuber girth, tuber length, leaf area, moderate to maximum stem diameter, few to average number of growing balls and average weight of vine. It has average to high percentage of diosgenin. The percentage of diosgenin varies from 3.45 to 4.43. The accessions (13,5,6,16) have more than 4% of diosgenin.

Complex III: was represented by a minimum of 5 rays and maximum of 10 rays and contains 5 accessions (8,12,11,2,1). The main characteristics were large stem diameter, maximum tuber length and girth, average leaf area and maximum number of buds. It has least to average percentage of diosgenin except in accession 12 which has a high percentage of diosgenin.

Table 12 — Index range and symbols used for different characters in *Dioscorea deltoidea*

Characters	Range of means	Score I		Score II		Score III	
		Value less than	Symbol	Value (range)	Sign	Value greater than	Symbol
Number of bines/ plant	2.25-22.50	9.25	0	9.25-16.25	ƀ	16.25	ď
Weight of vine/ plant (gm)	57.50-412.50	140.00	0	14.00-250.00	σ	250.00	σ
Diameter of stem (cm)	0 85-1.52	1.10	0	1.10-1.35	σ	1.35	σ
Length of tuber/ plant (cm)	10 82-43.45	22.00	0	22.00-32.00	Q	32.00	Q
Girth of tuber/ plant (cm)	17.80-66.07	31.82	0	31.82-51.00	ρ	51.00	ϙ
Number of buds/ plant/tuber	14.25-145.25	57.25	0	57.25-100.25	ϱ	100.25	ϱ
Number of growing balls	10.00-36.00	18.10	0	18.10-26.10	ϱ	26.10	ϱ
Leaf area/plant (cm^2)	5.61-21.72	11.00	0	11.00-16.00	ρ	16.00	ρ
Diosgenin %	2.90-4.93	3.57	0	3.57-4.14	ʋ	4.14	ʋ
Weight of tuber/ plant (kg)	0.06-4.00	1.77		1.77-2.68		2.68	
Number of Prominent bines/plant	1.25-10.00	2.75		2.75-4.50		4.50	

Complex IV: is represented by only one accession(4) and by 9 rays. It has moderate number of buds and the rest of characters are very high. The percentage of diosgenin is 4.42.

On comparing the complexes there is a marked difference between the complex I & IV, while most of the characters in I are represented by small rays, while in IV, it is represented by big rays. The maximum dissimilarity between complexes II & III were the % of diosgenin, stem diameter, girth and tuber length. Complex II seems to be intermediate between the complexes I and III. This study shows that *D. deltoidea* forms tentative four germplasm complexes into which accessions sort out themselves. This information could be utilized in breeding programme in order to combine characters from different germplasm complexes.

Harvesting of Tubers

Dioscorea deltoidea should be allowed to grow for a period of three years. Under Kashmir conditions the tubers should be harvested in the month of November. The tubers are deep and can be harvested by pick axe.

Storage

After harvesting, the tubers are cleaned with water and allowed to dry in open space under shade for couple of days. Then the dried tubers are stored in gunny bags at room temperature.

Pest & Diseases

No major pest and disease attack has been observed. However, during some field experiment it was observed that mortality rate was high. After digging out a large number of effected plants, it was observed that root rot had occurred due to the attack of different fungi namely *Pencillium* spp; *Alternania* spp and *fusarium* spp. After trying out different fungicides it was found useful to dip the rhizome cutting in 0.02% brassicol before plantation. This treatment forestalled the onset of such fungi resulting in root rot.

Also it was observed that in early May when the plants had obtained a height of about 1 metre, the plants would gradually show wilting of leaves, lose vigour and die. The investigation revealed that grub of an insect used to suck the sap of the plant just above the rhizome and within the ridge resulting in nearly 80% mortality rate.

Number of systemic and contact insecticides were tried. Finally endosulfan preparation (a contact insecticide) was seen to knock out the grub. Some aphids and mites used to attack the leaves of the plant in mid-June. This was controlled by prophylactic spray of insecticide (Thiodan 0.3%).

Chemical Analysis

A rapid spectrometric method was developed for the estimation of diosgenin using small quantities of *Dioscorea* tubers (Rishi *et al.* 1976). Pure diosgenin was isolated from *D. deltoidea* and further purified by preparative TLC using $SbCl_5$ solution

(24% in 70% $HClO_4$) for detection. 25 mg of pure diosgenin was dissolved in 250 ml of hexane (solution A) and 0.75ml and 1.0ml of this solution were taken in two tubes and the solvent removed by bubbling air. $HClO_4$(5ml) followed by 0.1ml of $SbCl_5$ solution (24% in 70% $HClO_4$) were added to each tube. The mixture were kept for half an hour. Absorbance was noted for both the solution (pink in colour) from 400 to 600 nm against reagent blank. Absorption maxima was noticed at 486nm. Volumes corresponding to 10 to 100 μg of diosgenin were taken from solution A into different tubes, solvent removed and colour developed as mentioned above. The absorbance of each was noticed at 486 nm against reagent blank and the complex obeyed Beers law.

Dioscorea tuber was dried at 105°C, finely powdered and passed through 72 mesh sieve. This material (50mg) and 25ml each of 3N HCl and hexane were refluxed in an all glass apparatus on a hot plate magnetic stirrer for 2 hours. The temperature was maintained between 90-96°C. The mixture was allowed to cool and the aqueous phase was given two successive shakings with 25ml of hexane and organic phase was washed with 1% $NaHCO_3$ solution and subsequently with distilled water and made up to 100 ml with hexane. Absorbance was noted at 486nm against the reagent blank. This method of estimating diosgenin has following plus points:

1. 8 to 18 samples can be analysed by one analyst in a day.
2. Small quantity (50mg) of the dried plant material is required.
3. Hydrolysis and extraction, being conducted simultaneously with the use of hot plate magnetic stirrer, is achieved in 1.5 to 2 hours.
4. Avoids additional solvent.

Intercropping

Taking into consideration the medicinal value of *Dioscorea deltoidea* in Pharmaceutical Industry and the importance of hops in brewing industry along with the fact that both are climbers, *Dioscorea deltoidea* was intercropped with hops, which not only helped in utilizing the space left between the hops plantation, but also helped in using the same trellis, irrigation and other agricultural operations complementing each others growth and also regenerating higher net returns/ha than *Dioscorea* or hops when grown alone. The lay out of the experiment is shown in the Figure 3. The method of plantation and other agricultural operations are discussed by Rishi *et al.*[33] The cost benefit ratio was highest viz. 1:1.61 when *Dioscorea* was intercropped with late cluster compared to 1:1.07 and 1:1.52 respectively when *Dioscorea deltoidea* and hops were grown alone. Such an intercropping was done for the first time and would also help in regenerating the fast depleting species of *Dioscorea deltoidea* which has been and is being indiscriminately extracted from the forests of North Western Himalayas.

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*Cultivation of *Dioscorea composita* Hemsl.: A potential source of diosgenin in Jammu

S. Gupta, Arun Kumar & S.N. Sharma

Regional Research Laboratory
Jammu - Tawi

GENUS *Dioscorea*, a monocot of family Dioscoreaceae with over 600 species is widely distributed in tropical world, a few species are temperate. About 15 species are known to contain steriodal sapogenins chiefly diosgenin in their underground tubers. Diosgenin is a versatile precursor for synthesis of a large number of steriod products such as Corticosteroids, sex hormones and antifertility compounds. *Dioscorea composita*, *D. floribunda* and *D. nipponica* are the most important commercially exploited source of diosgenin. They are central American and Chinese in origin. In India *D.deltiodea* & *D.prazeri*, occurring wild in North West and North East Himalayas respectively are the natural sources of diosgenin. According to one estimate the annual requirement of diosgenin in India is approximately 200 tonnes. The total production in the country is 25-30 tonnes annually and rest of the demand is met through imports in the form of finished products from abroad (Hussain, 1984)[1]. Recently other precursors like stigmasterol from soyabean oil and sitosterol from other plant sources have also contributed towards synthesis of these drugs in the world market. Inspite of this diosgenin still remains an important source particularly for production of corticosteroids (Hussain, 1988)[2].

Cultivation of exotic *Dioscorea*, in India became necessary in view of the depletion of wild material due to excessive collection by pharmaceutical companies, lack of efforts to regenerate it and also to help establish the Indian steroid industry based on indigenous raw material. As a result of collective efforts under "All India *Dioscorea* improvement project", *Dioscorea floribunda* is being suc-

*Revised and updated

cessfully cultivated as commercial crop around Banglore, Goa, Andamans and Assam, and *D.composita* in West Bengal, Tamil Nadu & Assam (Asolkar *et al.*, 1979)[3]. During the last 15 years or so many advances have been made in the field of propagation, agronomy, tissue culture and physiology of these species. This paper gives a resume of the work done in this direction on *Dioscorea composita* introduced at RRL, Jammu.

Propagation

Propagation of *D. composita* can be done by seeds, tubers or leaf node cuttings. In its original home (Mexico) it grows wild and is also raised in commercial plantations either by seeds or tubers. Propagation by leaf node cuttings is not practised on commercial scale because of high cost & labour (Gupta *et al.*, 1977)[4]

Propagation by Seeds

The seeds are sown in seed pansor polythene bags containing soil sand and farm yard manure in suitable proportions or coarse washed sand only. In either case the upper 1-2 cm layer is covered with vermiculite. Before sowing, the seeds are soaked in cold water for 3-4 hrs. The seed containers are kept in a glass house under diffused light. They start germinating in 2-3 weeks time and maximum germination is obtained after a month. Three to four months old seedlings are transplanted during rains i.e. July-August in well prepared ridges at 60 × 75 cm spacing. A mixutre of farm yard manure at 30-40 tonnes/ha and phosphorous at 150 kg/ha as superphosphate is applied as basic doze prior to transplanting. Nitrogen as urea at 300 kg/ha is applied in 2 split dozes. First application is made after a month of transplanting in September and second is given 6 months later. Potash is supplied at 150 kg/ha as muriate of potash alongwith urea.

Studies on seed germination (Table 1) have shown that seeds can germinate fairly well under Jammu conditions, during different times of the year. Maximum germination is obtained at 30°C March-April is the best season for sowing because this period is most suitable for further growth of seedlings in the field. Germination percentage is equally good from August - November but for large scale sowing this period is not recommended because of high rate of seedling mortality during winter which follows immediately (Sobti *et al.* 1982)[5].

Propagation from Tubers

Populations raised from seeds showed great variation with regard to tuber growth and diosgenin content. In order to obtain higher yields clonal multiplication of high yielding plants was very important. The clones were selected on the basis of good diosgenin percentage and high tuber yield.

Table 1 — Relative germination performance of *D. composita* Hemsl. seed during different times of the year

Source	Date of sowing	Media Soil/ petridishes	Time taken to germinate	% Germination
P.i.	Aug.-Sept.,1969	Petridishes	8 days	70
201783	,,	Soil	2-3 weeks	40
E.C.	Sept., 1970	Petridishes	8-10 days	65
87659	Sept., 1972	Soil	2-3 weeks	40
P.i.	Sept., 1972	Petridishes	8 days	60
201783	,,	Soil	2-3 weeks	40
Wyeth	Oct., 1975	Petridishes	10 days	75
Laboratories	March-April,1976	Petridishes	8 days	65
	,,	Soil	4 weeks	40
	Aug., 1976	Petridishes	8 days	57

Tuber Dormancy

Dioscorea composita tubers show strong dormancy which lasts from December to February and is a great handicap in large scale cultivation of this species. Under normal conditions tubers take 2-3 months to sprout and sprouting process is erratic and prolonged. At RRL a study was undertaken to find out various genetical and physiological factors responsible for causing dormancy. Involvement of endogenous inhibitor and its dynamics during different growth phases was investigated. The inhibitor has been identified as abscisin-II like compound (Gupta *et al.*, 1979)[6]. The crown portion of the tuber with preformed buds had a very low inhibitor content as compared to medians and tips. A correlation has been observed between concentration of inhibitor and dormancy. During dormant phase (Dec. - Feb.) inhibitor content is highest in tuber i.e 2.5 μg dry wt. It starts declining from April onwards with the advent of new sprouts and active growth, completely disappears in July and remains absent till November. Big tubers have higher inhibitor concentration compared to small ones. Temperature and humidity play a key role in breaking dormancy. Chilling, light and dark treatment were found ineffective. Several growth regulators like gibberellins, auxin, Ethrel, cycocel and morphactins were tried at various concentrations and different durations for breaking dormancy out of these only Ethrel has been found consistently effective. It has given increase in sprouting percentage over control and has also reduced the sprouting period, (Sobti *et al.*, 1982)[5].

Clonal variation in sprouting of *D. composita* tubers was found after studying sprouting behaviour in a number of clones over a period of 3-4 years. Although the age, time of harvest and environmental conditions at the time of experiment were

similar it was found that some clones sprout earlier have high sprouting percentage and shorter sprouting period as compared to others. Effect of storage date on sprouting of tubers was also noted. Under Jammu conditions tuber pieces can be stored from March to June. March-April storage is best as the miniplants so raised are right in time for field transplanting in July-August. Based on our studies a technique has been developed for mass production of *D. composita* from tuber pieces. It has given 70-80% sprouting in large scale experiments and has also shortened the time taken for complete sprouting. The technique is being practised till date for regeneration of this species.

Transplanting and After Care

Plants raised from tuber setts are transplanted in ridges either in March-April or during the rains i.e. July-August. They get established within a week. Plants need to be irrigated frequently when young, afterwards they should be irrigated once a week in summer and in winter as and when required. It is a hardy plant and can withstand drought conditions to some extent. During summer months Sprinkler irrigation was also used at Jammu.

Tissue Culture Method

For rapid clonal propagation of *Dioscorea* species, tissue culture technique has also been recommended. A number of workers (Laxmi Sita 1976[7], Mascarenhas *et al.*, 1976[9], Grewal & Atal 1976[10], Chaturvedi & Sinha 1977)[8] have reported to obtain complete plantlets in *D. floribunda* and *D. deltiodea* through tissue culture of various excised parts. Regeneration of *D.composita* plants from stem callus and nodal explants was reported by Datta, *et al.*, 1981[11], Grewal *et al.*, 1989)[12]. However this method is not adopted commercially inview of the high cost involved

Interspecific Hybridisation

Dioscorea is a dioecious plant. Female plants do not set seed unless male plants are in close physical proximity. Hand pollination or intertwining of male and female spikes has resulted in very good seed set at Bangalore in case of *D. floribunda* and *D.composita*. The latter also flowers profusely from September-January under Jammu conditions, but there is no viable seed formation. A number of factors may be responsible for this. It was observed that though pollen is 60% viable, pollen germination is low. Pollen tubes are very short and too slow to grow or reach the ovule. In order to stimulate germination and elongation of pollen tube, a number of *in vitro* systems were tried. Good pollen germination and tube elongation was achieved when pollen was germinated in a medium containing 0.15% sucrose solution in combination with 25 ppm cycocel followed by incubation at 28-30°C for 3-4 hrs, (unpublished data). But there was post fertilization ovular break down, in some cases embryo formation was observed but it could not proceed further beyond a few celled stage. Low temperature prevalent during this period (January) may be another factor responsible for seed sterility under Jammu conditions.

Work done at IIHR Bangalore has indicated that species within the new world group and within the old world group are cross compatible (Bammi *et al.*, 1979)[13] and between the groups the crosses are incompatible. In crosses between species of the new world group also, four levels of crossability were observed, which ranged from complete crossability to early break down of embryo to complete failure. Some promising hybrids between *D.composita* and *D.floribunda* have been made at IIHR Bangalore.

Spacing

Effect of population density on tuber yield and diosgenin content was studied under field conditions. Four inter and intra row spacings were tried i.e. 60 × 90 cm with plant population 18000/ha, 60 × 75 cm having 22,000 plants per hectare, 45 × 60 cm and 30 × 45 cm with 30,5000 and 46,000 plants/ha. respectively. The crop was harvested after 2 years and diosgenin content analysed (Table 2). It was found that with increase in spacing individual tuber/plant was increased but total yield/ha decreased. Highest tuber yield of 94.5 tonnes/ha (fresh) was found at 30 × 45 cm, but per plant yield was low i.e. 2.1 kg. Tuber yield per plant was maximum at 60 × 90 cm spacing i.e. 3.4 kg but total yield/ha was low in this case i.e. 57.8 tonnes. Diosgenin content was not affected by increase or decrease in spacing. This study has shown that 30 × 45 cm spacing is optimum for 2 year crop. But considering from diosgenin point of view, a 3 year crop with 45 × 60 cm spacing is recommended. Effect of population pressure on yield of *D. floribunda* has been studied by Sarma *et al.*, 1986[14]; Saxena 1985)[15].

Table 2 — Effect of population density on the yield and diosgenin content of *Dioscorea composita*

S. No.	Treatment (spacings) (cm)	Plant population/ha	Fr. tuber yield/ plant (kg)	Total tuber yield/ha Tons(fr.)	Total tuber yield/ha Tons(Dry.)	Diosgenin content% (DWB)
1.	T_1 (60×90)	18,000	3.4±.22	57.8	20	1.8±0.20
2.	T_2 (60×75)	22,000	2.7±0.19	56.7	18.2	1.4±0.13
3.	T_3 (45×60)	30,500	2.4±0.13	70.8	24	2.0±0.15
4.	T_4 (30×45)	46,000	2.1±0.10	94.5	30	1.8±0.17

Supporting System

Dioscorea vines need support for their maximum growth. The system tried successfully at Bangalore in case of *D. floribunda* consists of stone pillars 3m high

spaced 6 m apart in the field. G.I. wire No.6 or 8 is used on the four sides of the field interconnecting wires can be of 12 gauge. Each plant is supported by a gunny twine & tied to the over head wire. In case of *D.composita* stone pillars are replaced by G.I. pipes, rest of the structure is similar. Both of these supporting systems are cost heavy. Singh,R.S. (1982)[16] tried different methods of staking *D. floribunda* vines, they reported higher yields of dry tuber and diosgenin under the plants trained on rope fencing as compared to other methods such as bamboo, pigeon pea or castorbean staking. Simillarly Konnard and Norris (1968)[17] noted significantly higher yields in case of *D. floribunda* grown on the chicken wire Trellis pillar system than that grown on poles only or on dwarf castor bean or without support In search of a cheaper substitute for training *D.composita* plants at RRL, Jammu, three different supporting systems were tried. They were (i) the Wire-Trellis G I pipe system, Eucalyptus poles system and *Leucaena leucophylla* as live support, incase of control the vines were left trailing on surface and were pruned at 50 cm height periodically. Details of first two systems are similar to those described for *D. floribunda* except that stone pillars were replaced by G.I. pipes or Eucalyptus poles as the case may be. In *Leucaena leucophylla* type of support, seedlings of this species were grown in between *D.composita* plants and served as live supports, *Leucaena leucophylla* gets easily established and is periodically chopped, side branches are pruned, since it grows very fast and its roots fix atmospheric nitrogen also. This experiment was conducted at field station Chatha in 1 4 acre area in RBD with 2 replications. A perusal of the data shows that when *D.composita* is grown as a two year old crop on these systems, fresh tuber yield per acre in three systems viz. G.I. poles, Eucalyptus and *leucaena leucophylla* is, 196, 147 and 115 qtls respectively. Plants without support gave very low yield. The study indicates that the wire trellis- G.I. pole system has higher yield potential as compared to *Leucaena* or Eucalyptus type. But the cost of construction of this system has become formidable in view of the rising prices. Our experience at farm plantations of *Dioscorea composita* has shown that Eucalyptus pole — wire Trellis support system should be recommended for commercial cultivation of this species in order to reduce the cost. Economics of cultivation using this type of support have been worked out (information brochure 1993)[18] Leucaena type of support is useful for short term plantings only, since it competes with the crop and outgrows it with passage of time.

Nutrient Requirement

Dioscoreas in general have high requirement of Nitrogen. A field experiment was conducted to study the effect of different levels of Nitrogen, phosphorous and potash on yield and diosgenin content of *D.composita*. Results showed there was a significant and linear increase in tuber yield with Nitrogen upto 400 kg/ha. Phosphorous and potash were required in moderate quantity i.e. 150 kg/ha each, for optimum growth. The recommended fertilizer schedule for the crop is that

phosphorous and potash should be applied in single doze at the rate of 150 kg/ha each at the time of transplanting in pits alongwith Farm yard manure at the rate of 40 kg/ha. Nitrogen at 400 kg/ha has to be supplied in 2 splits coinciding with the active growth period of the crop i.e. first dose in April - May and second in Sept-Oct. of the same year.

Weed Control

During first and second year of growth the vines are weak and need great care. Periodic hand weeding as and when required is essential during this period. From third year onwards the overhead vines make a thick cover and provide shade as a result, weed population is considerably reduced and less weeding is required. Chemical weedicides such as Senecer at 3 kg/ha and Lasso at 3 kg plus Diuron at 3 kg/ha have been recommended in case of *D. floribunda* (Sobti *et al.*, 1986)[19]. Chemical weed control has not been reported in *D.composita.*

Pests and Diseases

No major pests or disease attack has been observed in case of *D.composita* under Jammu conditions. Some times cutworms and aphids were found to infest growing vines either in nursery or in the field. They can be controlled by 2% foliar spray of Sevin. Rarely fungus attack was seen on foliage but it did not cause any harm to the crop.

Intercropping

In case of *D. floribunda* intercropping with legumes like cowpea, horse gram, french bean and Mexican bean was found profitable. No such studies have been done in case of *D. composita.*

Harvesting

D. composita should be allowed to grow for a minimum period of three years. Though some high yielding clones selected at RRL, Jammu can also be grown as a two year crop economically. The diosgenin content also increases with age and is maximum during the third year. The tubers are deep and can be harvested by pick axes on deep ploughing.

Diosgenin Content and Tuber Yield

During an extensive selection programme for high yielding strains/clones in *D.composita*, mass screening of seed as well as clonal populations was conducted at RRL, Jammu. Table 3 shows wide variation in tuber growth and diosgenin content of plants in different age groups in seed population (S3). Fresh tuber weight/plant varies from 0.11 - 0.50 kg with diosgenin varying from Traces - 0.9%.

Table 3 — Diosgenin content and related yield attributes in different age groups in seed population of *Dioscorea composita* introduction no (S_3) at Jammu (1979)

	1 Year		2 Year			3 Year			4 Year		
*Tuber yield (kg)	Diosgenin %	Percentage hyd mass	Tuber yield (kg)	Diosgenin %	Percentage hyd. mass	Tuber yield (kg)	Diosgenin %	Percentage hyd. mass	Tuber yield kg	Diosgenin %	Percentage hyd. mass
Range 0.11-0.50	Trace to 0.92	–	0.85-2.75	1.2- 3.2	8 0-29.4	1.2-8.2	1.2-4.9	8.3-20	2.0-24.05	1.1-3.9	10.25
Mean 0.27	0.61	–	1.5	1.9	15.1	4.2	2.7	14.2	6.9	2.3	17.1
S.K. 0.06	0.084	–	0.12	0.080	1.21	0.45	0.20	0.74	1.2	0.14	1.0

*Mean 20 plants

Table 4 — Tuber yield and diosgenin content at different harvests in clones of *D. composita*

Clone No.	Wt. of mother tuber (fresh) (kg)	Diosgenin (%) in mother tuber	12 month		24 months		36 months	
			Tuber yield/ plant (kg)	Diosgenin (%)	Tuber yield/ plant (kg)	Diosgenin (%)	Tuber yield/ plant (kg)	Diosgenin (%)
S_2(10)	10.50	2.6	0.5	2.0	1.8	2.1	3.6	2.4
S_2(23)	20.0	2.7	0.55	2.4	1.9	2.7	5.25	3.6
S_2(27)	8.75	2.6	0.45	2.0	2.0	2.1	5.5	2.4
S_2(28)	10.0	3.1	0.45	2.2	2.3	2.3	5.0	3.0
S_2(44)	10.0	2.8	0.55	2.0	1.7	2.8	4.26	2.7
S_2(58)	24.05	4.0	0.50	2.0	3.06	3.6	6.5	4.1
S_2(69)	15.0	2.5	0.46	2.0	1.50	2.6	3.50	2.5
S_2(72)	8.0	3.5	0.30	1.8	1.1	2.8	3.1	2.5
S_2(53)	11.08	3.2	0.50	2.0	2.1	3.0	4.1	2.8
*S_3(115)	4.9	5.0	0.55	2.2	1.7	4.8	3.26	4.9

*High yielding clones selected for multiplication

when seed crop is harvested after 12 months growth. During 2nd year tuber yield varies from 0.85 2.7 kg with diosgenin 1.2 - 3.2%. During 3rd and 4th year there is significant increase in tuber weight on an average 4.2 kg fresh tuber/plant in 3rd year & 6.9 kg after 4 years growth was obtained. Thus there exists a linear relationship between age and tuberisation in case of *D composita*. Some tubers as big as 35 kg (fr. wt) were found in 4 year old seed crop. As regards diosgenin content it increases during the first 2 years of growth and thereafter in 3rd year get established at a particular level depending upon the genotype.

A comparative study was made in case of clonal population too. During first year rate of tuber growth is slow and fresh tuber weight per plant varies from 0.3 - 0.55 kg (Table 4). After 3 years it becomes very fast i.e. 3.2 - 6.5 kg/plant. However on closer examination it was found that lot of variation exists as regards tuber yield per plant in case of clonal populations too, inspite of the fact that they were grown under uniform conditions of soil fertility, irrigation and initial weight of tuber piece planted. It appears that tuberisation process is an interplay of many factors. Clonal populations compared to seed populations of the same age were found to be superior in diosgenin content. The latter is a highly stable character in a clone and remains so even after 2-3 generations (Table 5). Recently some clones were harvested after 15 years of selection and subsequent regeneration inorder to analyse their diosgenin content. It was found that diosgenin percentage in them was same as reported in their mother tubers. Field trials with selection S_2 (58) were conducted at Chatha Farm RRL and economics of growing it as 3 year old crop worked out (Information Brochure 1993)[18]. Dry tuber yield of 48- 50 tonnes/ha with 3.6% diosgenin was obtained when plant population is 30,000/ha. With closer spacings high yielding selections like S_2 (58) and S_3 (115) with 4-5% diosgenin and faster growth rate can also be economically grown as 22 year crop. They have been released for multilocational trials and reported to have given much higher yields under agroclimatic conditions of Maharastra (Thane),West Bengal and Assam (Pers. Comm)

Table 5 — Tuber yield and diosgenin content in selected clones of *D. composita*

Clone No.	Fresh wt. mother tuber at harvest (kg)	Diosgenin (%)	Ist generation three-year old crop		2nd generation three-year old crop	
			*Tuber yield/ plant (kg)	+Diosgenin (%)	Tuber yield/ plant (kg)	Diosgenin (%)
S_2(58)	24.05	4.0	6.5	4.4	6.0	3.8
S_3(115)	4.9	5.0	3.26	4.9	5.25	4.8

*Mean 30 plants
+Mean 10 plants

Physiological studies

Distribution of Diosgenin during Different Times of the Year

A number of clones of uniform age (3-4 yrs) were selected for this purpose. Diosgenin content in tubers was analysed month wise following Selvaraj (1971)[20] for one year starting from April. It was observed that diosgenin content does not show much variation during different times of the year. In month of April plant resumes active growth after dormancy, diosgenin content is low during April - May. In June it picks up, during July-August there is a little decrease but from September onwards it reaches the same level as in mother tuber (Fig. 1); from December-March it is stable and tubers are harvested during this period. Datta *et al.* (1984)[21] have reported maximum accumulation of diosgenin (3.6%) in tubers of *D. composita*, followed by shoots and leaves (from lower portion) 1.6% middle portion 0.039% and upper 20 nodes 0.03% (d.w.b.) Chatterjee *et al.* (1979)[22] reported that defruiting operation in *D. prazeri* and *D.composita* affected the diosgenin content favourably

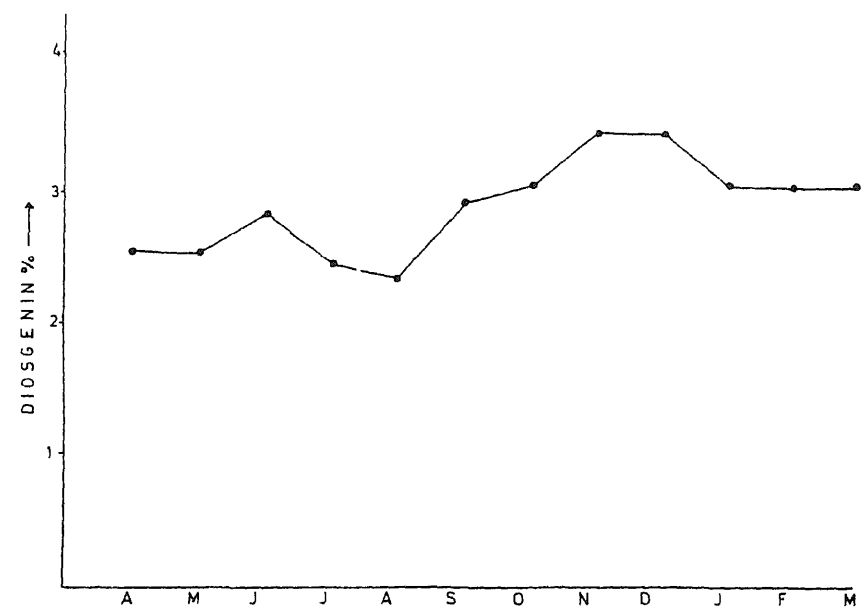

Fig.1 Distribution of diosgenin in clone S_2 (58) during different times of the year.

when supplied with Nitrogen, potash and bacterial fertilizers than without defruiting Mandal and Chatterjee (1985)[23] also conducted histochemical and biochemical studies in *D.composita* in relation to its diosgenin content.

Effect of Position of Planting Piece

It has been observed that position of planting piece on mother tuber influences tuber yield in the long run (Bammi *et al.*, 1979)[13]. Depending on position, a tuber can be divided into 3 sections : (1) crown with preformed buds, (2) Median, and (3) Tip without any buds. Though genetically alike these sections behave differently physiologically. Crowns are earlier to sprout whereas medians and tips take longer time, because buds have to be formed new in them during storage. This can be one reason that plant raised from crowns out - yield the other ones. In *Dioscorea composita*, the study was conducted with 2 clones. They were raised from crown, median and tip portions separately and grown under uniform field conditions. After one year the growth and yield parameters were recorded. Crowns showed highest tuber yield followed by median and tip sections, whereas the plants raised from a mixture of crowns, medians and tips were lowest in yield Leaf area (cm^2) was high in plants raised from crowns Diosgenin content did not show any significant difference amongst the plants raised from different sections of the tuber

Effect of Shade

The effect of shade on growth attributes viz. vine height and weight, number of leaves/plant, leaf area, leaf area index (LAI) and tuber weight/plant was studied in *D.composita* grown at Chatha Farm of RRL, Jammu. All the parameters showed a positive relationship with tuber yield which was significantly higher under partial shade as compared to natural light (Gupta *et al.*, 1990)[24]. Reduction in light intensity, however did not affect diosgenin content of the plant.

Physiological Analysis of Growth

Work on quantitative analysis of yield variation in medicinal *Dioscorea* species is limited. In *Dioscorea composita* various physiological parameters which may influence growth and yield in this species were identified. For this purpose a number of genotypes were screened for characters like specific leaf weight (SLW), tuber weight, chlorophyll and diosgenin content. Two clones differing in yield potential were also selected for studying characters like LAI, SLW, RGR, NAR and CGR at different stages of growth and development following Radford (1967)[25]. In case of genotypes, it was found that higher specific leaf weight is invariably associated with high tuber weight (unpublished data). Genotypes having higher SLW also possessed high chlorophyll content as compared to those with low SLW. Diosgenin content on the other hand does not show any relationship either with high SLW or tuber yield. This study reveals that thick leaf possesses high chlorophyll content and is related with higher biomass.

Clones

In *Dioscorea composita*, during first year growth is very slow, it catches in second year. Hence one year old clones were selected for growth analysis study. Growth period was divided into 5 phases): (i) period of active growth, (ii) Flowering and fruiting; (iii) dormancy, (iv) resumption of new growth , and (v) vegetative period. A perusal of the data presented in Tables 6 and 7, shows that specific leaf weight was high during dormancy and resumption of new growth as compared to other stages. It showed significantly positive correlation with biomass yield, ($r = 0.6435$) and positive association with LAI ($r = 0.2255$) and dry tuber weight ($r = 0.4320$) leaf area index showed significant positive relationship with CGR, ($r = 0.5175$) fresh and dry tuber weight and negative correlation with RGR and NAR. Leaf area index for both clones increased from period of active growth to dormancy and then started to decline till harvest. Net assimilation rate (NAR) recorded high value during flowering and fruiting stage and then started to decline. Crop growth rate (CGR) increased during period of active growth, flowering and fruiting, started to decline in dormancy and resumption of new growth, again moved up in vegetative period. Total dry matter increased in both the clones consistently during all the stages of growth. Rate of tuberisation on the other hand was slow during period of active growth, it increased fast during post flowering and dormancy. At resumption of new growth it was stable but decreased during vegetative period. The pooled analysis showed that high yielding clone S_2 (58) was superior in all the characters compared to S_3 (87). On the basis of results discussed, it is clear that SLW, LAI, CGR and biomass yield can be used as important markers in selection of high yielding genotypes/clones in *Dioscorea composita* (Sharma *et al.*, 1991)[26].

Effect of Growth Regulators and Micronutrients

A number of growth regulators like cytozyme crop^{+}, Ethrel and Gibberellic acid were tried as foliar spray for increasing yield potential in *Dioscorea composita*. None of these has given any significant increase in tuber yield or diosgenin content under field conditions.

"Micron C" a micronutrient formulation comprising Cu, B, Zn, Fe, Co, Mn, Mb, S, Ca and Mg at 0.5% solution when given as foliar spray twice a year increased tuber growth significantly, both in one year and 2 years old clonal crop. On unit area basis an increase of 60-70% in fresh tuber yield over control was recorded (Table 8) Diosgenin content however remained at par with control. The treated crop was luxurious, healthy, foliage being dark and lush green and tuberisation rate increased (Gupta *et al.*, 1982)[27].

Post Harvest Treatment for yield Improvement

Genetic upgrading, clonal selection and tissue culture approaches have been adopted to increase diosgenin content in *Dioscorea* species. Use of growth regulators (Vasantha Kumar 1980[28], Nandi & Chatterjee 1982)[29] and radiations (Gupta,

Table 6 — Physiological indices for yield potential in clones of *Dioscorea composita*

Stage of growth	Months	Physiological parameters													
		Vine length/ plant (m)		Fresh tuber wt. (kg)		S L W gd m^{-2}		L A I		R G R gg^{-1} d^{-1}		N A R gdm^{-2} d^{-1}		C G R g/day/plant	
		$S_2 58$	$S_3 87$	$S_2 58$	$S_3 87$	$S_2 58$	$S_3 87$	$S_2 58$	$S_3 87$	$S_2 58$	$S_3 87$	$S_2 58$	$S_3 87$	$S_2 58$	$S_3 87$
Period of active growth	July	2.95	2.94	0.162	0.160										
	Aug	3.79	3.27	0.294	0.209	0.59	0.57	1.68	1.43	0.025	0.018	0.044	0.030	0.074	0.043
	Sept	3.96	3.50	0.366	0.293	0.52	0.49	3.30	2.42	0.014	0.007	0.0390	0.0138	0.0719	0.0333
Flowering and Fruting	Oct	5.16	4 36	0.655	0.374	0.52	0.40	4.88	3.33	0.0151	0.0124	0.0430	0.0171	0.1099	0.0569
	Nov	5.40	4.70	1.500	1.000	0.55	0.47	7.76	5 58	0.0376	0 0439	0.0515	0.0560	0 3996	0.3012
	Dec	6.15	5.37	2.200	1.470	0.57	0.53	7.68	6.07	0.0111	0.0096	0.0242	0.0263	0.1858	0.1596
Dormancy	Jan	6.68	5.38	2.230	1.540	0.69	0.65	8.40	6.14	0.0029	0.0024	0.0110	0.0044	0 0924	0 0182
	Feb	7.10	5.80	2.370	1.620	0.71	0.56	6.45	5 41	0.0027	0.0024	0.0135	0.0102	0 0870	0.0555
Resumption of new growth	March	6.68	5.25	2.180	1.520	0.62	0 54	4.78	3 60	0.0019	0 0018	0.0143	0.0130	0 0683	0.0468
	April	7.64	6.50	2.000	1 380	0.71	0.45	4.79	2 86	0.0016	0.0010	0 0108	0.0120	0 0517	0 0514
Vegetative period	May	8.30	7.10	2.140	0 983	0.54	0.39	4.57	3 85	0 0011	0 0014	0 0094	0 0097	0 0424	0 0373
	June	9.00	8.5	2.00	0 960	0 55	0 40	4.60	3 90	0.0010	0 0009	0.0070	0 0050	0 0362	0 0231

Table 7 — Correlation coefficient for different characters in two clones of *D. composita*

Characters	Vine length/plant	Fresh tuber wt/plant	Dry tuber wt/plant	Biomass yield/plant (Total dry matter)	SLW	LAI	RGR	NAR	CGR
1	2	3	4	5	6	7	8	9	10
Vine length/plant	I	0.8201**	–0.0089	0.3270	0.5040*	0.2760	–0.2330	–0.1800	–0.1450
	II	0.8073**	–0.0089	0.2370	0.2042	0.3420	–0.4601	–0.1552	–0.1787
	III	0.8116**	–0.0089	0.2820	0.3541	0.3090	–0.3466	–0.1675	–0.1615
Fresh tuber wt/plant	I		0.9673**	0.7563**	0.5344*	0.6516*	–0.6080	–0.7860	–0.0385
	II		0.8710**	0.8770**	0.5344*	0.7954*	–0.5981	–0.2412	0.0961
	III		0.9190**	0.8165**	0.5344*	0.7230*	–0.6030	–0.5135	0.0576
Dry tuber wt/plant	I			0.9290**	0.4475	0.4149	–0.2915	–0.8794	0.1300
	II			0.8319**	0.4176	0.6245*	–0.4915	–0.2935	0.1228
	III			0.8800**	0.4320	0.5190*	–0.3910	–0.5860	0.1260
Biomass yield/plant (Total dry matter)	I				0.5896*	0.2484	–0.8027	–0.9902	–0.1870
	II				0.6986*	0.5134*	–0.7801	–0.6508	–0.0319
	III				0.6435*	0.3805	–0.7910	0.8200	–0.1090
SLW	I					0.3768	–0.6262	–0.2035	–0.2380
	II					0.0752	–0.5317	–0.1136	–0.1165
	III					0.2255	–0.5785	–0.1580	–0.1770

1	2	3	4	5	6	7	8	9	10
LAI	I						–0.011	–0.0101	0.4270*
	II						–0.3119	–0.0315	0.6086*
	III						–0.1610	–0.0205	0.5175*
RGR	I							0.9305**	0.3780
	II							0.9563**	0.8743
	III							0.9430**	0.6260**
NAR	I								0.6112**
	II								0.9077**
	III								0.7590**
CGR	I								
	II								
	III								

I = $S_2 58$, II = $S_3 87$ clone, III = Combined analysis of $S_2 58$ & $S_3 87$ Clones.
*Significant at 5% (P = 0.05) **Significant at 1% (P = 0.01)

Table 8 — Effect of Micronutrients on tuber yield and diosgenin content on clones of *D. composita*

Clone	Age	Treatment & conc.	*Tuber yield plant (kg)	**Diosgenin (%) D.W.B.	Mean tuber weight/ plot (kg) ($33m^2$)
$S_2(52)$	1 Yr 6 months	Control	1.040±0.59	3.1	–
$S_2(52)$	,,	Micron C–O 5%	1.770±1.14	2.8	–
$S_2(52)$	2 Yrs 6 months	Control	3. 36±2.48	3.10	80.70
$S_2(52)$	-do-	Micron C-O 5% ***	5.51±3.45	2.70	144.75
C.D. at 5%			2.06		
$S_3(46)$	1 Yr. 6 months	Control	0.65±0.67	2.8	–
$S_3(46)$	-do-	Micron C-O 5%	0.870±0.45	2.5	–
$S_3(46)$	2 Yrs 6 months	Control	1.36±0.77	2.90	45.75
$S_3(46)$	-do-	Micron C-O 5%	***2.36±1.08	2.40	75.30
C.D. at 5%			2.01		

* Mean 20 plants.
** Mean 10 plants.
*** Significant at 5% level.

M.N. 1984[30]; Banerjee *et al.*, 1984[31]; Sahoo *et al.*, 1985[32] and Dixit *et al.*, 1985)[33] was also made for this purpose in case of *D. floribunda*. But diosgenin yield could not be increased to any desirable extent by these methods. Search for more effective and economical method for yield improvement continued. In 1982 a fungal strain RLA-5 of *Aspergillus* was isolated from naturally infected tubers of *Dioscorea composita* growing in experimental fields of RRL, Jammu (Gupta *et al.*, 1988)[34] This strain upon inoculation to fresh and healthy tubers and subsequent incubation, increased their diosgenin yield considerably compared to control. This opened up the possibility of its large scale utilization. In order to establish that only RLA-5 strain of *Aspergillus* is the causal agent for increase in diosgenin yield in treated tubers, inoculations with other sources like water alone, culture media, (Potato dextrose broth) autoclaved RLA-5, and inoculum (RLA-5) filterate were also tried and their effect observed. Data shows (Table 9) that inoculation with none of these sources was effective except RLA-5 liquid culture and its filterate where 50% and 20% increase in diosgenin yield over control was noted. Thus efficacy of this strain in augmenting diosgenin yield was confirmed. Further efforts were directed towards perfecting a suitable method of inoculation in large scale operations. Different methods of inoculation were tried (Table 10). A perusal of the data shows that inoculation of solid culture by bore method of whole tubers gives a fairly good yield of diosgenin compared to control, but process is laborious and time consuming. Application of liquid culture in suspension to very small tuber pieces is not effective. Similarly increase in diosgenin with slurry method is insignificant. Liquid culture shaker method gives significant increase but it is less than that obtained by Needle board method. In shredding experiments some increase was obtained after

Table 9 — Effect of culture media, water autoclaved inoculum and inoculum filterate on diosgenin yield in tubers of clone S_258

S.No.	Treatment	Dry matter	Diosgenin (%)	Diosgenin yield in 1 kg (gm)	Net increase over control (%)
1	C	20	3.2	6.4	–
2	W	16	4.0	6.4	–
3	M	14	4.4	6.1	–
4	A	16	3.8	6.08	–
5	X	16	6.0	9.6	50
6	F	14.8	5.2	7.6	20

C - Control
W - Inoculation with distilled water
M - Inoculation with medium only
A - Inoculation with autoclaved fungi
X - Inoculation with RLA-5 liquid culture
F - Inoculation with RLA-5 liquid filterate only.

Table 10 — Effect of different methods of inoculation by RLA- 5 on diosgenin content (%) in tubers of *Dioscorea composita* clones.
(Period of treatment July 1984; values are mean of 3 replicates on dry wt. basis)

S. No.	Mode of Inoculation	S_257			S_258			S_3115		
		O	RLA-5	% Increase over control	O	TLA-5	% Increase over control	O	RLA-5	% Increase over control
1.	Solid culture bore method	2.7	6.2	130	3.1	6.2	100	3.2	6.4	100
2.	Liquid culture needle board method	2.7	9.5	251	3.1	9.0	190	3.2	8.8	175
3.	Slurry method	2.7	3.6	33.3	3.1	3.3	6.4	3.2	3.6	12.4
4.	Shake method	2.7	5.2	92	3.1	5.3	71	–	–	–

72 hrs incubation at 35°C. But this increase was also not significant. Hence, for large scale treatment of tubers only inoculation by Needle board method with liquid culture of RLA-5 to whole tubers pieces (200-300 gms) has been found highly effective. Various parameters which may affect the activity of this fungus were also studied. These were: (1) different incubation temperature, (2) varying periods of incubation after inoculation; (3) different concentrations of inoculum (4) age of culture; (5) seasonal effect on activity of RLA-5, and (6) Effect of RLA-5 Treatment on diosgenin yield of tubers from different age groups, bulk collections etc.

Incubation Temperature

Three incubation Temperatures i.e. 15°C, 25°C and 35°C were tried where as incubation period was kept constant for 15 days. Fresh tuber material of a single clone was divided into lots of equal weight. These were inoculated with RLA-5 liquid culture and incubated at 15°C, 25°C and 35°C respectively. Another 3 lots were maintained as controls at respective temperatures. The experiment had 3 replications.

Incubation Period

Here incubation temperature was kept constant, but incubation period was varied from 5, 10, 15 to 20 days. Rest of the details were similar as mentioned above.

Inoculum Concentration

Different concentrations of inoculum (RLA-5 liquid culture) *viz.*, 0.5, 1.0, 1.5 and 2% respectively were tried for inoculation. Incubation temperature and period were kept constant i.e. 35°C and 15 days respectively. The infected tubers were extracted for diosgenin after the specified period.

Age of Culture

Culture of different age groups *viz.*, 15 days, 1,2 and 3 months old was tried for inoculation of fresh tubers of a single clone.

Seasonal effect on RLA-5 Activity

In order to study activity of RLA-5 in a particular clone throughout the year, comprehensive experiments with (20 kg lots) clone S_2 (58) were set up every month for a period of one year, controls under identical conditions were maintained. For inoculation, standard liquid culture needle board method was followed. Net increase in diosgenin calculated on per kg basis of the material.

Effect of RLA-5 on Mixture of Clones

In commercial collections of *Dioscorea*, tuber material is a mixture of number of plants varying in age, source and origin. In order to observe the effect of RLA-5 treatment on such collections, tuber material from different clones growing at laboratory Farm was pooled and subjected to this treatment.

Figure 2 shows effect of incubation temperature on RLA-5 activity. It was found that increase in temperature enhances the activity of RLA-5 and a maximum increase of 48% over control in diosgenin yield was obtained at 35°C, Figure 3 shows that after inoculation with fungus RLA-5, incubation of tubers for 5-10 days is insufficient as evidenced by insignificant increase in diosgenin yield over control. Incubation for 15 days is optimum since highest increase of 44% is noted at this period. Further increase in incubation period decreases the yield. Variation in quantity of liquid inoculum used for inoculation affects the production of diosgenin. It was observed (Fig. 4) that increase in inoculum concentration directly increases the diosgenin production upto 2% level, lower dozes of 0.5 & 1% gave less yield. Therefore 2% inoculum was considered optimum for maximum diosgenin production. Age of initial culture also plays a vary important role. In order to have maximum effect the culture should be physiologically young and active. For this purpose parent inoculum should be properly maintained and regular subculturing followed. It was found that culture was quite effective from 15 days upto 2 months of subculturing and gave substantial increase in diosgenin yield over control there after it begins to lose its potency.

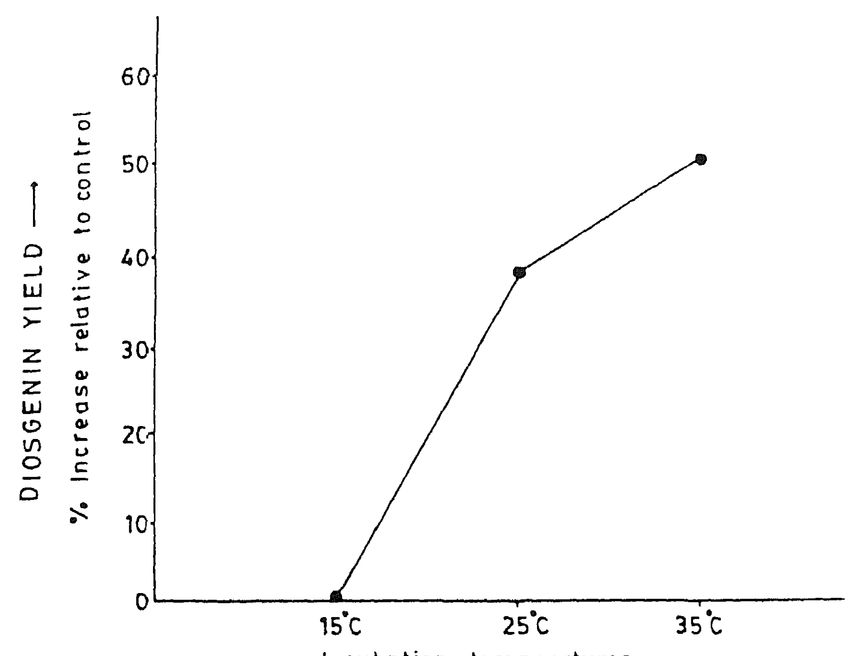

Fig. 2 Effect of different temperatures on activity of RLA-5

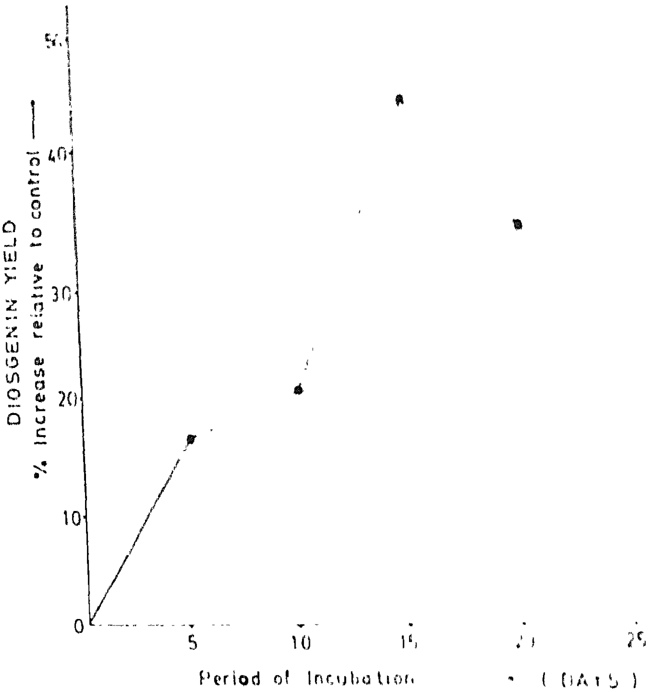

Fig 3 Effect of different incubation periods on activity of RLA-5

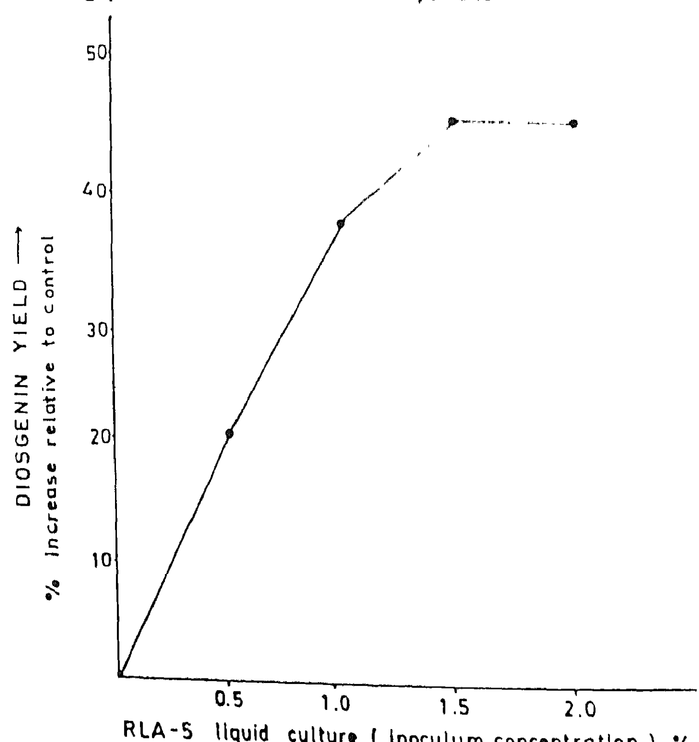

Fig. 4 Effect of inoculum concentration on activity of RLA-5

Table 11 — Effect of RLA-5 treatment on diosgenin yield of *D composita* in a single clone during different times of the year. Clone: $S_2(58)$

S. No.	Period of treatment	Dry matter (%)		Diosgenin obtained in 1 kg material (gms) (DWB)		Net increase over control (%)
		Control	RLA-5	Control	RLA-5	
1.	Jan.	25.0	24.0	7.5	9.1	21
2.	Feb.	25.0	15.0	6.8	9.2	35
3.	March	25.0	20.0	7.5	10.4	38
4.	April	24.0	22.0	6.0	8.5	40
5.	May	22.0	19.5	5.8	8.4	44
6.	June	24.5	12.6	6.6	9.7	47
7.	July	23.0	15.3	6.4	10.3	60
8.	Aug.	25.0	12.5	5.8	8.7	50
9.	Sept.	23.0	14.5	6.6	9.4	42
10.	Oct.	20.0	13.2	6.0	9.0	50
11.	Nov.	20.0	14.2	8.0	10.8	35
12.	Dec.	23.0	21.4	7.8	10.2	30

Seasonal effect on activity of RLA-5 liquid culture has been studied separately in a single clone throughout the year. Table 11 shows that from March - November a net increase of 40-60% over control in diosgenin yield was obtained. In winter months i.e. dormancy (Dec. Jan & Feb.) increase is less.

When a number of tubers from different clones or genotypes were pooled and bulk material subjected to this treatment, a net increase of 48% in diosgenin yield was obtained. This methodology has been standarised upto bench scale level (30-40 kg tuber lots). From this study the optimum conditions for the process worked out to be Temp. 35°C, incubation period 2 weeks inoculum concentration 2% and age of culture 15 days — 2 months old.

In literature there are some reports on increase in diosgenin by incubation of tubers in *Dioscorea*. Selvaraj *et al.*, (1980, 1982)[35-37] have found increase in yield on incubation of fresh tuber homogenates at a particular temperature for a specified time or by feeding crude enzyme extract to dry homogenates of *D. floribunda*. According to them the increase in diosgenin is caused by endogenous or exogenous enzyme systems. They have also demonstrated the presence of such systems in actively growing tuber portions and leader shoots of *D. floribunda*. Some reports on the role of fungi in stimulating diosgenin level are also available. Krider *et al.*, (1954)[38] have reported that "saponases" which are hydrolysing enzymes (converting saponins to sapogenins) are also produced adaptively by several species of fungi when grown in presence of Saponins. It is well known that diosgenin occurs as glycoside dioscin in plants. Dioscin on hydrolysis yields diosgenin. Nagai *et al.*, (1970) have reported that enzymatic hydrolysis with *Aspergillus terreus* in tubers of *Dioscorea tokori* increases yield of diosgenin from 90-120% as compared with known method of acid hydrolysis. Increase in yield of diosgenin has also been reported in *Tribulus terrestris* by enzymatic treatment with a preparation of *Aspergillus niger* (Perepelitsa *et al.*, 1978, 1979)[40-41]. Present studies with RLA-5 strain of *Aspergillus* also lend support to the possibility that when this fungus comes in contact with fresh tubers of *D.composita* and grows upon it, during the period of incubation, it may possibly be releasing those enzymes which cleave dioscin to diosgenin thereby enhancing the diosgenin production.

Acknowledgements

The authors are grateful to Director, Regional Research Laboratory, Jammu, for his keen interest in this work. Thanks are also due to Mrs. Navin Kapoor for diosgenin analysis of tuber samples. Assistance of Sh. Madan Lal in conducting various experiments and recording field data is gratefully acknowledged.

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*Saprophytic Production of Ergot Alkaloids

Hans-Peter Schmauder

Otto-Schwarz-Str
59, D-07745 Jena, Germany

Introduction

THE pioneering work of Prof. Arthur Stoll in Basle (Switzerland) more than 50 years ago was a landmark in ergot research and paved the way for many discoveries within this type of fungal metabolites. There are some routes to prepare the ergoline skeleton synthetically, but no method is economically feasible. Therefore, the production of ergot alkaloids relies at least partially on biological procedures.

In principle following possibilities exist for the production of ergot alkaloids.

1. Isolation from the sclerotia of *Claviceps purpurea* Fr. (Tul.) which may be obtained by parasitic cultivation.
2. Extraction from saprophytic cultures of different ascomycetes preferably *Claviceps* species.
3. Partial synthesis (the ergoline skeleton is obtained biologically and e.g. the peptide portion is prepared synthetically and combined with lysergic acid moiety).

The era of saprophytic production was inaugurated by the fundamental work of Prof. Abe in Japan shortly after the Second World War (see for instance: Abe 1951a,b; Abe *et al.* 1952, Abe & Yamatodani, 1964). He examined ergot fungi, parasitic on wild grasses, from Japan and other parts of the Far East. During this work the first culture medium was developed which is especially suited for saprophytic production of ergoline alkaloids.

*Revised and updated

Some advantages of saprophytic production compared with the field cultivation are obvious. For example, saprophytic production is not dependent on the weather and the production can be performed throughout the year. Methods for isolation, cultivation, selection and conservation of such strains will be reviewed in the present paper.

Methods for Isolation and Selection

Sclerotia are the starting material for the isolation of strains able to produce alkaloids under saprophytic conditions, which is described in many patents (e.g. Rutschmann & Kobel, 1963a, b; Amici *et al.* 1967 C; Molnar & Tyihak, 1970; Wack *et al.* 1973; Kobel & Sanglier, 1975). The different species of *Claviceps parasitize* on particular hosts. *C. purpurea* and several related species are spread throughout the world. They favour pasture grasses and cereals (e.g. rye, wheat and barley) as hosts. Sclerotia of *C. purpurea* mostly contain alkaloids of the peptide-type (e.g. ergocristine, ergocornine, ergokryptine, and ergotamine). Strains of *C. fusiformis* grow on *Pennisetum* species in warmer regions of the world (e.g. Central Africa). Their sclerotia synthesize in most cases clavine-type alkaloids. A third important species is *C. paspali*, which grows on Paspalum in various parts of the world (e.g. the southern parts of Europe, America and Asia). Sclerotia of these strains contain commonly simple derivatives of lysergic acid (For more details see Mantle, 1975; see also Fig. 1).

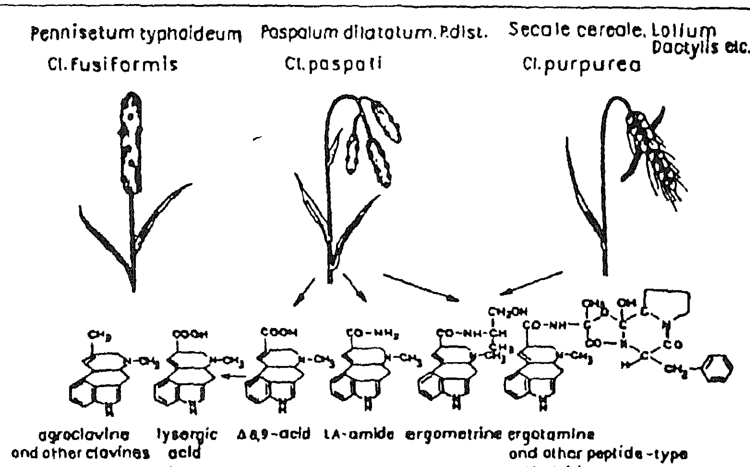

Fig. 1—Examples for hosts and alkaloid spectra of some *Claviceps* species (from Kobel, 1971)

For isolation of saprophytic producing strains the sclerotia of high alkaloid content have to be sterilized in a controlled manner, e.g. as described by Tyler (1963), Gröger and Tyler (1963), Abe and Yamatodani (1964), Kobel *et al.* (1964), Amici *et al.* (1967c) and Eich (1973). The surface of the sclerotia may be treated with mixtures of equal parts of alcohols (e.g. ethanol, *n*-propanol) with water, solutions of formaldehyde in water (about 5%), or solutions of mercury salts (e.g. 5% $HgCl_2$ in water). After sterilization a piece from the inner tissue of a suitable ergot is placed on an agar slant for growth. After a few days or weeks the fungi grow around this piece and the resulting mycelia and conidia have to be carefully transferred to another agar slant. The media used in these steps are commonly rich in nutrients. Kobel *et al.* (1964) have used malt extract or beer wort agar for *C. paspali.*

After a few passages on one of these rich media the strains have to be tested under standardized conditions for their ability to produce alkaloids in saprophytic culture. Mostly these strains are able to produce a little amount of alkaloids in the initial passage. But they lose this ability quickly. In most cases the first mycelium grown on the agar medium is white and has a lot of aerial mycelium. Only few of the colonies show morphological differences and a partial change in pigmentation. These differences are dependent on the species of Claviceps used. It is reported that strains of *C. purpurea* which are able to produce alkaloids mostly show a typical pigmentation wavering between beige, red and purple (Udvardy-Nagy *et al.* 1964; Molnar *et al.* 1964; Borowski *et al.* 1972; Kobel & Sanglier, 1975). The colonies of such strains show also a typical morphology. They are often sharp-edged, conical emerging and free of aerial mycelium. Media used for cultivation and selection are less rich in nutrients. The named rich media commonly are employed for isolation from sclerotia and in some cases for production of conidia. The media for cultivation on agar dishes or slants suitable for a selection step, by reason of morphological characteristics, are usually synthetic. These contain only in some cases small amounts of complex natural compounds (e.g. beer wort, malt extract, yeast extract). As a source of carbon generally sucrose, mannitol, sorbitol are used and additionally an ammonium salt of a Krebs- cycle acid or ammonia as a source of nitrogen and mineral salts are added.

Desai *et al.* (1986) describe that Asparagine is a stimulus of the alkaloid production in shaking cultures. Rozman *et al.* (1985) have used soybean peptones of the types III and IV (Sigma) as N- sources to some purpose. With *C. fusiformis* CF 13 higher yields of clavines were observed than after an application of meat peptones. Ludwigs *et al.* (1985) have found that some strains of Claviceps are able to use urea as a sole source of nitrogen for production of alkaloids. For a prevention of bacterial contaminations Kren *et al.* (1986) have applied some broad-spectrum antibiotics, e.g. chloramphenicol, streptomycin, oxytetracycline, and tetracycline to cultures of *C. fusiformis* W1. Chloramphenicol was the best antibiotic which had no negative effect on alkaloid formation.

The data on special selection procedures are rare. Due to the economic importance of Claviceps details are not disclosed. In general, it is necessary to develop for each strain special methods for selection and stabilization.

In some patents special ways for selection of strains are described.

Udvardy-Nagy *et al.* (1964) have cultivated a strain isolated from sclerotia on agar media containing only starch or dextrin as a source of carbon Colonies showing a violet, purple or orange-violet colour and a typical morphological pattern were selected and tested in saprophytic culture for their ability to produce alkaloids. Further cultivation of patent strains is continued on media containing glycine as a source for nitrogen.

The *p*H value of the used agar media (6.5-6.8) is outside of the normal range. The liquid culture broth contains in this case 0.01 - 0.3% glycine also.

Chain *et al.* (1960a) have selected a strain of *C. paspali* by inoculation of spores on sterilized rye embryos. The developed sclerotia-like structures were used for further selections. They have found selectants able to produce simple lysergic acid derivatives.

In an other patent Wack *et al.* (1973) have isolated a strain of *C. purpurea* (OKI 88/1972) which is able to produce ergocornine and ergokryptine as main alkaloids from a sclerotium with high alkaloid content. The strain was selected after repeated passages over special nutrient media containing ammonium nitrate in increasing concentrations (1.0 to 10.0 g/l). The test for its ability to produce alkaloids took place in liquid culture media containing 0.5g KH_2PO_4 and up to 50 g sodium chloride/l.

Only in few cases it is possible to select a saprophytic producing strain from sclerotia with success. Mostly the isolated strains lose the ability for alkaloid production when they are cultivated saprophytically in contrast to the parasitic culture. Kobel *et al.* (1962) have compared the productivity of parasitic and saprophytic strains. The qualitative alkaloid spectra were nearly identical in the sclerotia and in the corresponding saprophytic cultures. While the quantitative distribution differed considerably.

There are also problems with chemical races described by Kobel *et al.* (1964) and Gröger (1975).

Special ways for a selection of alkaloids producing strains of Claviceps have described Gaberc-Porekar *et al.* (1983) and Pertot *et al.* (1986). For the first paper an application of UV light for the selection procedure was developed using an addition of a fluorescent indicator, e.g. fluorescein or 2′, 7′-dichloro- fluorescein, to the solid medium and than a detection of the alkaloids in the colonies by UV light. But in the correct *p*H range a detection of peptide-type alkaloids directly in UV is possible.

In the second paper the authors write upon a selection of strains after γ-irradiation of a high-production mycelial strain. After the mutation step a strain with conidiation was selected. But it had a significantly lowered alkaloid productivity.

The Degeneration Phenomenon

As stated above the saprophytic alkaloid producers in the genus Claviceps have a typical morphology and in most cases a characteristic pigmentation. This typical feature depends on different outer and inner influences e.g. the culture state, the age, the degeneration phenomenon and the culture broth. Often a close correlation between the "sclerotia-like" form of the culture and the ability to produce alkaloids is mentioned.

Only the sclerotial type of colonies produce synnematic pellets in submerged cultures and forms alkaloids. In the case of *C. paspali* a group has found this type of colonies only to be the dominant in strains derived from sclerotia (Vero *et al.* 1966). An analogous system was described by Tonolo and Udvardy-Nagy (1968). Only the sclerotial form of the strain B 35 used is able to produce alkaloids. The authors had found also a close correlation between colonial morphology and ability for alkaloid production. These heterokaryotic systems can split easily and the strains are more unstable.

Above all, Italian scientists have postulated a heterokaryotic stage as a pre-requisite for a satisfying and stable alkaloid production. The heterokaryotic strains are distinguished by forming "giant-colonies". For *C. purpurea* it is described, that the "producers" have characteristic morphological properties, e.g. they are heterokaryotes ; e.g. strains FI 275, FI 32/17, FI 540, FI 43/14 and FI 82. It is postulated that alkaloid production in saprophytic culture is generally bound to the heterokaryotic conditions of producing lines (Spalla *et al.* 1969; Spalla, 1973 ; Spalla & Marnati, 1979).

A discussion of the problem of sectored colonies of a high yielding *C. purpurea* strains give Didek-Brumec *et al.* (1991).

On the other hand, there are also homokaryotic strains able to produce high amounts of alkaloids in saprophytic culture. These strains do not form "giant colonies". For instance there are the strains *C. fusiformis* SD 58 (Gröger, 1959). *C. purpurea* Pepty 695 (Gröger & Erge, 1970; Erge *et al.* 1972), *C. purpurea* DM 838 (IMET PA 136) (Borowski *et al.* 1972, Breuel *et al.* 1979); *C. purpurea* Pepty 695/ch (Maier *et al.* 1980); *C. purpurea* Ech k 420 and Exy 20 (Kobel & Sanglier, 1975, 1979); *C. paspali* Li 342/SE 60 (Gröger, 1965).

For many of the high-producing heterokaryotic as well as homokaryotic strains a susceptibility to degeneration is observed. Segal & Germanier (1974) have described an appearance of degenerative morphological change by the pigmentation for a strain of *C. paspali*. During this process the morphology and pigmentation of the parent strain changed remarkably. The white colonies show mostly a decrease

of alkaloid synthesis. By continued selection of this strain it was possible to conserve the ability for alkaloid production. Similar results are also described by Kobel (1969).

Likewise strains of *C. purpurea* show a tendency to degenerate (Kobel & Sanglier, 1979). Crossing to the wild-type forms was also observed in this species.

On the other hand the characteristic morphology of producing strains (e.g. pigmentation and growth forms of colonies) can be obtained by adding of "morphogenic substances" to the culture media for cultivation of wild-type strains free of alkaloids in saprophytic culture. These strains can reduce the content of aerial mycelium, can grow in typical sharp-edged, partial conical emerging colonies, can

Table 1: Selection way for two strains for claviceps purpurea, a summary of results described by Kobel and Sanglier (1975)

	C. purpurea Ech k 420	*C. purpurea* Exy 20
(1)	(2)	(3)
Starting material	Sclerotia from rye (Wisconsin/ USA)	Sclerotia from Festuca pratensis (Rhone-Valley)
1st mutation step	Ethyleneimine	UV
Killing rate	99%	90-95%
No. of tested colonies	8,000	3,000
Increase of productivity	230%	120%
2nd mutation step	Sodium nitrite	Ethyleneimine
Killing rate	99%	99%
No. of tested colonies	10,000	9,000
Increase of productivity	100%	100%
3rd mutation step	NNMG	0.01 M bis-chloroethyl-methylamine
Killing rate	97%	Not determined
No. of tested colonies	10,000	12,000
Increase of productivity	125%	75%
4th mutation step	UV	
Killing rate	99%	
No. of tested colonies	10,000	
Increase of productivity	95%	
Alkaloid production in mixed culture	Both produce altogether 779 mg/l in equal parts of all alkaloids in the mixture.	
Main alkaloid(s) isomer	Ergocristine, ergokryptine and their isomers	Ergocornine, its isomers

produce the typical dark red or purple colours under these growing conditions. But they cannot synthesize alkaloids in spite of this induced sclerotia-like growth e.g. by addition of coffeine, theophylline (each 10 m*M*) or cysteine (0.5%). There is not a dependent correlation between sclerotial growth and alkaloid production (Schmauder & Gröger, 1979).

The Improvement of Strains by Mutation

In various papers and patents the use of mutation techniques is described. Mostly a combination of mutation and selection is used. Favourable for mutational experiments is the presence of conidia. In many strains there is an inverse relationship between the alkaloid production and their ability to form sufficient conidia. In some of the cited patents the absence of conidia is mentioned. In such cases, it is necessary to carry out mutation experiments with mycelial fragments. But this possibility is problematic for the interpretation of the results.

If the conidia are present, all variants of mutations are possible. On the one hand different radiations are used, in most cases UV or X-rays. The other great group is the induction of mutagenesis by chemicals.

The technical methods correspond to the usual ones. Also Claviceps spp. show a tendency for DNA repair in the daylight after UV- radiation.

Some of the known chemical mutagens applied are besides others.

1. N-nitro-N′-nitroso-N-methylguanidine (NNMG),
2. Ethylmethansulfonate (EMS),
3. Methylmethansulfonate (MMS),
4. Ethyleneimine and
5. Nitrous acid.

Also in these cases the common methods are used.

The results reported in some papers and patents may differ from each other. This will be illustrated with some examples: Segal & Germanier (1974) have used ethyleneimine or UV-radiation for treatment of *C. paspali*. An enhancement of white colonies which is a poor culture type regarding alkaloid synthesis, is reported In an other example Kobel and Sanglier (1975,1979) have isolated two important strains by a combination of mutation and selection. By this way the yield of alkaloids has been raised many times. In two examples Kobel and Sanglier (1975) described the selections of *C. purpurea*, Ech k 420 and Exy 20, from sclerotia able to produce saprophytically ergocristine and ergocornine/ergokryptine and their isomers, respectively By combination of different mutation steps with a selection of several thousands of colonies it was possible to increase the alkaloid production manifold (see Table 1). In other cases it was shown that there is the possibility for a change of the alkaloid spectrum

Kóbel and Sanglier (1973) have reported the change of a *C. purpurea* strain able to produce ergocornine and ergokryptine in ergocristine strains after treatments with mutagens. They have used UV-light, NNMG,EMS and methyl-bis (chloroethyl)amine with success as mutagens in the experiments from which the ergocristine strains were isolated.

Gröger *et al.* (1978) isolated wild-type strains of *C. purpurea* from a sclerotium rich in ergotamine. These strains are free of alkaloids under saprophytic conditions. After two mutation steps (UV-light) a strain of *C purpurea* (MUT 168) was selected able to produce alkaloids in saprophytic culture. This strain is not able to synthesize ergotamine, the main component in the sclerotium. It synthesizes a mixture consisting of 90% ergosine/ergosinine and 10% clavines in saprophytic culture. Consequently on one side it was possible to convert by mutation a non-producing one into a producing strain. On the other side, the alkaloid spectrum was completely changed. Another change of alkaloid spectrum after mutations is described by Marnati *et al.* (1975). In this example a producer for 6-methyl-$\Delta^{8,9}$-lysergic acid proceeded from an ergocristine strain.

An other system of different subsequent mutation steps for isolation of high producing strain describe Kren *et al.* (1986). Starting from a tetracyclic-clavine-producing strain (*C. purpurea* 129/35) they have isolated after a 3-stage UV-mutation strain 59 producing on to 6.5 g alkaloids/l consisting of 40-60% chanoclavine-I, 20-38% chanoclavine-I-aldehyde, 10-15% elymoclavine, and 5-10% agroclavine.

The Maintenance of Strains

After isolation, selection and/or mutation of strains it is very important to conserve the ability of saprophytic production. For conservation there are four possibilities available.

1. Continuous passages over agar media
2. Lyophilization with protective colloids
3. Preservation over or in liquid nitrogen, and
4. Preservation in other systems at temperatures below - 20°C.

Generally the strains show a tendency to degenerate by continuous transferring from slant to slant. In most cases permanent selection is necessary. This method is cumbrous and the productivity after several passages is not guaranteed in each case

The second method is more practicable with spore-forming strains. In this case the spores were suspended in a colloid (e.g. with milk-sucrose-solutions) or mixed with silica gel or an other similar product. After lyophilization the tubes were sealed and stored at room temperature, at 4°C or in a deep freezer (Mizrahi & Miller, 1968). Pertot *et al.* (1977) have some non-sporulating Claviceps strains lyophilized,

too. After three years of storage at 4°C all strains have shown all interesting biochemical properties and a good vitality.

The third possibility is described by Kelleher and Botnick (1969). Mixtures containing the mycelium was cooled under controlled conditions to temperatures near - 75°C before the closed ampoules were stored in a liquid nitrogen refrigerator. After more than one year the productivity was in the same range of the starting culture. This procedure is also practicable with non-sporulating Claviceps strains.

In a special way Kobel *et al.* (cited by Mizrahi & Miller, 1968) have used a combination of these procedures. Freezed cultures grown on beer wort/corn steep liquor agar were stored at - 70°C. A fourth method was described by Bassett *et al.* (1973). To a preculture (7 days old) glycerol was added and samples (approximately 1.5 ml) of these mixtures were transferred into ampoules which were flame sealed. These ampoules were cooled at a rate of about 1°C/min to - 70°C and transferred to liquid nitrogen. In this paper a storage on agar slants is not mentioned.

The strains

The saprophytic alkaloid producing strains are the pre-requisite for production of ergot alkaloids. A classification of such strains is possible with respect to their biochemical capacities. Starting from the alkaloid content some groups of strains are worth-mentioning (see also Gröger, 1979a,b; Spalla & Marnati, 1979):

1. Clavine forming strains, e.g. *C. fusiformis* B 35, *C. purpurea* Pepty 695/ch, *C. purpurea* strain 129, *C. fusiformis* SD 58.
2. Strains forming simple lysergic acid derivatives, e.g. *C. paspali* NRRL 3027, *C. paspali* Li 342/SE60.
3. Strains forming peptide-type alkaloids. They can be divided in some sub-groups.

3.1 Ergotamine strains, more than 2/3rd of the formed alkaloids is ergotamine in combination with its isomer ergotaminine, e.g. *C. purpurea* ATCC 15 383, *C. purpurea* FI 275, *C. purpurea* OKI 56/1970.

3.2 Ergosine strains, e.g. more than 80% ergosine/ergosinine are in the alkaloid mixture formed by *C. purpurea* MUT 168.

3.3 Ergocristine strains, e.g. *C. purpurea* ATCC 20 103 (IMI 131 669, FI S 40), *C. purpurea* Ech k 420.

3.4 Ergocor-nine and ergosine forming strains, e.g. *C. purpurea* FI 43/14.

3.5 Ergocorn and ergokryptine forming strains, e.g. *C. purpurea* FI 32/17, *C. purpurea* IC/39/20.

3.6 Ergocor-nine and ergokryptine forming strains, e.g. *C. purpurea* Exy 20, *C. purpurea* OKI 00022, *C. purpurea* DM 838 (IMET PA 130), *C. purpurea* Pepty 695.

TABLE 2 A Survey of Alkaloids Producing Claviceps Strains*

Species	Special designation	Type of culture	Alkaloid content	Main alkaloid	Other alkaloids	References
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Agropyran-type ergot		e c	~600	I(80)	II, V	Abe, M. 1945, 1946, 1948 cited by Abe and Yamatodani, 1964 Abe *et al.*, 1952.
Aspergillus(??)		s.c		VII		Siegle & Brunner, 1963
Sphacelia sorghi (taxonomy not clear)		e c.	700	XIV a	II, V	Mantle, 1973
C litoralis		e c.	~107	XIII (63)	V	Abe *et al.*, 1952, 1955; cited by Abe and Yamatodani, 1964
Claviceps sp.	B 4, B 5 strain 231	e.c. (s c)	up to 1000-1500	I, III	II, V	Stoll *et al.*, 1954a, b and Hofmann *et al.*, 1957
Claviceps sp.	15 B, 47 A	s c	up to 1270		V	Brady and Tyler, 1960
Claviceps fusiformis	SD 58	e c (s c.)	~800	III I	II,V	Groger, 1959
Claviceps fusiformis		s.c		III(65)	I,II,IV,V	Borella *et al.*, 1967
Claviceps fusiformis	B 35	s c	1600		V	Tonolo & Udvardy- Nagy, 1968

(*Contd.*)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Claviceps fusiformis	139/2/1	s.c	f. ≥ 3000 (up to 6000)	I	V	Banks *et al.*, 1974
Claviceps fusiformis		s.c e c	170	III (65)	I,II,V	Singh and Hussain, 1977
Claviceps fusiformis	00211	s.c., f.	1815	III(1630)	I (240)	Trinn *et al.*, 1984
Claviceps fusiformis	CF 13	s.c.	2,180	III (60-80%)	I	Rozman *et al.*, 1985
C paspali	F 550 (and f., other ones)	s.c.	1800	VI	V	Arcamone *et al.* 1960, 1961
C paspali	ATCC 13 892 (F-140) ATCC 13 893 (F-S/13/1) ATCC 13 894 (F-237) ATCC 13 895 (F-240)	s.c. f.	1000-1600	VI	VII,XV	Chain *et al.*, 1960 a-c
C.paspali	NRRL 3027	s.c.,f.	800	VI (87)	I-IV, IX, XV	Rutschmann and Kobel, 1963 a, b
	NRRL 3081	s.c.,f	400	IX (78)	II-IV, VI, XV	
	NRRL 3082	s.c.,f.				

(Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
C.paspali	ATCC 14 988	s.c.	420	n.m.	V, VI, VII	Tyler, 1963
C.paspali	NRRL 3080	s.c.,f.	500-650	VIII (86)	V, VI, IX	Rutschmann *et al.*, 1963
C.paspali		s.c.	600-1400	VI or VIII	V, XV, IX	Kobel *et al.*, 1964
C.paspali	Li 342/ SE60	s.c	400	II(40)	V	Gröger, 1965
C.paspali	Nr. 2 etc.	s.c.	more than 1000		V,VI	Mary *et al.*, 1965 Pacifici *et al.*, 1962, 1963
C.paspali	PA-me	s.c.	420	II, IX (Each 30)	V	Wack *et al.*, 1966
C.paspali	NRRL 3167 (from NRRL 3080	s.c., f.	2480-3330	VIII	V	Kobel and Schreier, 1966
C.paspali	47 A (HLX 123)	s.c.	1000		V,VI	Vining, 1970
	H 60 IV (HLX 509)		130			Vining and Nair, 1966
C.paspali	NRRL 3166 (from NRRL 3027)	s.c.,f.	2210-2270	VI(80-90)	I-IV, IX	Rutschmann and Kobel, 1967
C.paspali	F 2058	s.c		VI	V	Mercantini *et al.*, 1967
C.paspali	Ta 1	s.c	1056		V-VII, IX XII, XV	Brar *et al.*, 1968

(*Contd.*)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
C.paspali			1500	VI/VII		Sastry *et al.*, 1979
C.paspali	DSM 2836	s.c	525	XIII(α-)		Sastry, 1979 Wilke & Weber, 1984 a
C.paspali	DSM 2837	s.c	390	II		Wilke & Weber, 1984 b
C.paspali	MG-6	s.c.		VI		Bumbova-Linhartova *et al.*,1991
C.paspali		s.c.	336		IX (26%) VI (20.5%) VII(8.5%)	Janardhanan & Hussain, 1990
C.purpurea		e.c.	~138	XVI	I, II V, IX	Abe *et al.*, 1959, 1960; cited by Abe and Yamatodani, 1964
C.purpurea		e.c. s.c.		X XI-XIII		Kybal *et al.*, 1961
C.purpurea,	OKI 620 125	e.c. s.c.,f. s.c. f.	480	IX, XIII (30 each) XII (20)		Molnar and Tetenyi, 1962
C.purpurea			~585	VI, VII		Amici *et al.*, 1963
C.purpurea		s.c.	110-540	XII-XIII,IX (both together 60)	XV, V	Udvardy-Nagy *et al.*, 1964

(Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
C.purpurea,	ATCC 15 383 (F-1317/3)	s.c.,f.	900-1450	X		Amici *et al.*, 1964
C.purpurea,	OKI 22/1963	f.	300	XI (30)	V,VII,IX	Molnar *et al.*, 1964
C.purpurea,	F I 275	s.c.,f	1500	X (80)		Amici *et al.*, 1966, 1967a, Arcamone *et al.*, 1970
C.purpurea,	ATCC 20 019 (F I 101a)	f.,s.c	100-1500	XIII		Amici *et al*., 1967c
C.purpurea,	IMI 131 509 (F I 32/17)	s c.,f	1200-3800	X (50) XIII (50)		Amici *et al*., 1968a
C.purpurea,	ATCC 20 106 (F I 43/14)	s.c.	950-1100	XII (60) XIV (40)		Amici *et al*., 1968b
C.purpurea,	PRL 1980	s c	~1000	I (55) III (30)	II, V	Hsu & Anderson, 1969
C.purpurea,	F I 82	s c (?)	150-200	II		Spalla *et al*., 1969
C.purpurea,	Pepty 695	e.c.	≥ 900	XII, XIII (sum 50)	II, IX, V VII	Gröger & Erge, 1970 Erge *et al*., 1972
C.purpurea,	OKI 56/1970	e c s c	1300-1850	X (80)	V, VII, IX	Molnar and Tyihak, 1970

(Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
C.purpurea,	IMET PA 130 (D-M 838)	s.c.,f	2470	XII (sum 52) XIII	V IX, XV	Borowski *et al.*, 1972 Breuel *et al.*, 1979
C.purpurea,	OKI 88/ 1972	s.c.,f.	1700	XII, XIII (sum 50-70)	V, IX, XIV	Wack *et al.*, 1973
	OKI 00022	f.	1100	XII (65) XIII (30)	XI (5)	Szarvady *et al.*, 1972
C.purpurea,	IC/114/IRN IC/117/2	s.c.	500	X	XV	Bassett *et al.*, 1973
C.purpurea,	IC/39/20	s.c.	1100-1300	X (52)	I, II, III, XII, XIII	Galeffi *et al.*, 1974
C.purpurea,	Echk 420 Exy 20	s.c., f. s.c.,f.	780-950	XI (1) XII, XIII(1)	XV	Kobel and Sanglier, 1975
C.purpurea,	F.I. 231	s.c.	1200	XIII (3)	(30%)	Bianchi *et al.*, 1976
C.purpurea,	Strain 129	s.c.,f.	≥4000	I (92)	V, XV	Rehacek *et al.*, 1977
C.purpurea,	MUT 168	e.c. s.c.	350	XIV (90)	V(10)	Groger *et al.*, 1978
C.purpurea,	S 40 T	s.c.	700	XI	XV	Beacco *et al.*, 1978 a (2)
C. purpurea	ATCC 15383 ATCC 20103 ATCC 20019	s.c.		X XI XIII		Beacco *et al.*, 1978 b (2)
C.purpurea,	Pepty 695/ch	s.c.	500	II (80)	V	Maier *et al.*, 1980

(Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)
C.purpurea,	CCM F-725	e.c.	250	XII (α- ,β-) 1:1 to 2:1		Strnadova *et al.*, 1982
C.purpurea,	129/35, strain 59s.c.	6.500	II (40-60%)	III, I, V		Kren *et al.*, 1986
C.purpurea,	MNG 00186	s.c.,f		XII XIII(β)		Wack *et al.* 1981
C.purpurea,	L-17	s.c.	up to 1,200	XII, XIII	XIV	Didek- Brumec *et al.*, 1991
C.paspali	L-52	s.c.	1,500	VI	VII	Rozman *et al.*, 1989
C.purpurea,	1029c	e.c.		VI, XV	X,VII	Trejo Hernandez *et al.*, 1993
C.purpurea,	ATCC 20 103 (FIS 40)	s.c.	1,000	XI		Minghetti *et al.*,1968
C.purpurea,	PRL 1578	s.c. or e c. (?) 35 d	29-77	IX, X, XIV	V, XI, XIII	Vining & Taber, 1963

(Contd.)

TABLE 2 Continued

The synthesized alkaloids are indicated in the following manner

I	Agroclavine	X	Ergotamine/ergotaminine
II	Chanoclavine	XI	Ergocristine/ergocristinine
III	Elymoclavine	XII	Ergocornine/ergocorninine
IV	Penniclavine	XIII	Ergokryptine/ergokryptinine
V	I-IV and other clavine alkaloids	XIV	Ergosine/ergosinine
VI	Lysergic acid amides/isolysergic amide	XIVa	Dihydroergosine
VII	Lysergic acid/isolysergic acid	XV	Other alkaloids
VIII	6-methyl-$\Delta^{8,9}$ ergolene-8-acid	XVI	Peptide - type alkaloids
IX	(ergobasine/ergobasinine) ergometrine/ergometrinine		

Other symbols are:

n.m. not mentioned
s.c. submerged culture
e.c. emerged culture
f. cultivation in fermenters
d. days of cultivation

If a strain is cited in various papers, one paper is given as an example.

The approximate maximum content of alkaloid is given in mg/l culture broth.

In some cases the content of the main alkaloids is given in per cent in parentheses.

(1) Commonly cultivated in mixed culture

(2) Experiments with feeding of antimetabolites or substituted amino acids, which are precursors of alkaloid synthesis for obtaining of pharmacologically active alkaloid derivatives. For these experiments it was necessary to select mutants auxotroph against non-hydroxylated amino acids, e.g. leucine or phenylalanine.

(3) Only the β-isomer is synthesized.

3.7 Dihydroergosine forming strains, e.g. Sphacelia sorghi (Mantle, 1973).

For more details see also Table 2.

In contrast to saprophytic non-producing ones the producing strains are distinguished by the following characteristics

(a) The mycelium structure and morphology. The morphology is described above (see also Vorisek & Rehacek, 1976, Vorisek *et al* 1974) The mycelium is mainly swollen short and strongly granular

(b) The ability of spore formation. Generally the spore formation and the ability of alkaloid production under saprophytic condition are reciprocal (e.g. Pazoutova *et al.* 1977).

(c) Lipid content of the cells is much higher. It might be in some cases correlated to the formation of alkaloids. The lipid formation and alkaloid synthesis very often occur concomitantly but do not strictly depend on each other. Besides lipid formation other differentiation steps are necessary to allow alkaloid formation.

(d) The activities of some enzymes important for the alkaloid biosynthesis are strongly regulated. The main enzymes of tryptophane biosynthesis are of higher activity than in non- producers and show another profiles in the activity during the fermentation (Schmauder & Gröger, 1973). Enzymes of the alkaloid pathway are present only in cell-free extracts of producers (Erge *et al.* 1973; Maier & Gröger, 1976).

The strains show differences in relation to alkaloid secretion or storage Most of the strains contain all (Molnar & Tetenyi 1962) or a part of the alkaloids within the cells (Amici *et al* 1967c). Only a few of the strains secrete all the alkaloids in the culture liquid (Borowski *et al.* 1972).

The Cultivation Techniques

For production, selection or spore formation different compositions of culture broths are necessary. The media for isolation or spore formation are mainly rich in nutrients. They contain mostly complex natural mixtures, e.g. beer wort, corn steep solids, corn steep liquids, plant meals or yeast extract.

For selection or production of alkaloids mainly synthetic media consisting of sources for carbon, nitrogen and mineral salts were used. As sources for carbon generally sucrose, glucose, mannitol, sorbitol or similar sugar alcohols are used. Sucrose concentrations between 5 and 30% are mentioned. In relation to the sugar content an addition of a sugar alcohol in parts three to one can cause a stimulation of the alkaloid synthesis. As a source of nitrogen ammonia, asparagine, urea, ammonium nitrate or ammonium salts of some di- or tricarboxylic acids (e.g. citric, succinic, malic, maleic or oxalic acid) are mostly applied.

The absolute necessary mineral salts with range used are KH_2PO_4 (0.02-0.2%), $MgSO_4$ * 7 H_2O (0.02-0.08%), in some cases $FeSO_4$ * 7 H_2O (1-100 mg/l) and $ZnSO_4$ * 7 H_2O (10-100 mg/l) are also used. For *C. purpurea* also KCl (0.012-0.015%), $CA(NO_3)_2$ (0.1%), in some cases KNO_3 (2-3%) or NaCl (0.5-5%) are useful. For more details see Arcamone *et al.* (1961). Vining and Nair (1966), Brar *et al.* (1968), Spalla *et al.* (1969) and in the relevant patents. In some cases an inhibition by higher phosphate concentrations is described. The optimum is near 0.025-0.05% (see also Gröger & Tyler, 1963; Arcamone *et al.* 1970; Robbers *et al.* 1972). A slight activation is described with addition of Zn^{++} - and Fe^{++} - ions. In some cases (e.g. for *C. fusiformis* SD 58, or strains of *C. purpurea*) the alkaloid synthesis is induced by addition of tryptophan or other tryptophan derivatives to the liquid culture broth (e.g. Floss *et al.* 1974; Brar *et al.* 1968).

The fermentations are done in the temperature range of 20° -30°C (favourable 23°- 26°C) and in a *p*H range of 4.6-6.3 (at best 5.2- 5.8).

Two possibilities for fermentation are stated. Firstly, an emergic culture is possible. In this variant the fungi grow on the surface. The cultivation time is in most cases longer than 3 weeks. The yield in relation to the volume and the cultivation period is mostly also unfavourable. The culture vessels are commonly Erlenmeyer-, Fernbach-flasks or other ones. Vlcek & Kybal (1975) and Kybal & Sikyta (1986) have described a culturivation in plastic bags, which can be thrown away after the cultivation, or other equipments for emergic culture. In this system a profitable production is imaginable because the plastic bags are easy to pile up and spare working time used for cleaning etc. after the cultivation of *C. purpurea* 1029c in solid state culture on wheat grains.

Secondly, there is the submerged culture in shaking flasks or fermenters. For industrial use, this variant is in most cases only applicable with profit. The optimal cultivation time is between 7 and 21 days. An important role plays the rotary frequency for shaking cultures. The mainly used conditions are 180-240 rpm. For cultures in fermenters the aeration rate and the construction of the stirrer and the chicanes are decisive.

The following problems are most characteristic for the Claviceps fermentation:

(a) In most cases the bulk of alkaloids are located within the mycelium.

(b) The fermentations of Claviceps require very high standards of sterility.

(c) The strains are sensitive against small alterations in fermentation conditions.

(d) The strains are commonly also sensitive against mechanical stress and variations in the aeration and stirring.

(e) The highest potent strains are mostly free or poor in spores. Difficulties in conservation of the strains without loosing of their productivity are well-known.

For more details refer papers by Abe and Yamatodani (1964), Amici *et al.* (1967a, b), Arcamone *et al.* (1970), Kelleher (1970), Thomas and Bassett (1972), Gröger (1972). Banks *et al.* (1974), Arcamone (1977), Spalla (1973) and Spalla & Marnati (1979).

In contrast to *C. fusiformis* and *C. purpurea*, which do not need special preculture conditions, most of the strains of *C. paspali* require special techniques for preparing the inoculum. These strains can only produce alkaloids after a precultivation in a richer medium containing corn steep or corn syrup products or similar mixtures. It is necessary to use the inoculation regime agar slant or dishes - preculture in a richer medium - production media (see also Tyler, 1963; Gröger & Tyler, 1963; Kobel *et al.* 1964). Contrary to this species the other ones can produce alkaloids also by a direct inoculation in synthetic media (production media, e.g. *C. purpurea* Pepty 695 - Erge *et al.* 1972) or a similar medium with a little amount of yeast extract and a higher - level of phosphate for a better growth and higher yield of biosynthetically active biomass (e.g. *C. fusiformis* SD 58 - Erge *et al.* 1972). The isolation, separation and purification of the synthesized alkaloids can be done in the same manner as it is known for other alkaloids. In some of the cited patents or reviews these operations are described in detail.

Concluding Remarks

Apparently in some cases the saprophytic production of ergolines is more economical than the parasitic cultivation on rye plants. Alkaloid yields in fermenters, as a percentage of the mycelial dry weight, may exceed 20%, whereas natural sclerotia contain less than 1% or even less than 0.1%. In submerged culture or fermentation the process is more precisely controlled, the cultivation is not limited to the specific season and the production and isolation of alkaloids can take place on the same site. Furthermore, it is possible to apply some manipulations which are not practicable under field conditions (e.g. feeding of special substances, such as inducers, substrates, analogues, etc.) (Mantle, 1975).

A detailed knowledge about physiological particularities and interactions between different metabolic part of the cellular pathways are of increasing interest for reaching higher yields of alkaloids and for the directed formation of modified alkaloids with better properties and effects especially in the medical application. On these topics a very high number of papers and reviews are published. Because the length of this review is limited a concise summary of topics and the most interesting published papers is given (in a short selection):

Overviews: Robbers (1984), Kobel & Sanglier (1986), Rehacek & Sajdl (1990), Kren (1991), Schmauder (1992), and Socic & Gaberc- Porekar (1992).

Effect of 5-fluoro-tryptophan and other antimetabolites on growth and alkaloid formation: Skinner *et al.* (1967), Pazoutova *et al.* (1990), Schmauder (1992).

Regulation of metabolism ("general" and specific regulation): Pazoutova *et al.* (1982 & 1987), Pazoutova & Rehacek (1984), Desai *et al.* (1986), Socic *et al.* (1986), and Puc *et al.* (1987).

Interactions between fatty acid metabolism, sterol metabolism and alkaloid formation: Kren *et al.* (1985a,b), Pazoutova *et al.* (1988).

Physiological control, process kinetics and mathematical modelling of growth and alkaloid production: Pazoutova *et al.* (1981), Milicic *et al.* (1987), and Flieger *et al.* (1988).

Application of immobilized strains of Claviceps for alkaloid production (mostly in a laboratory scale) and studying physiological properties of protoplasts and of the strains: Kopp & Rehm (1984), Komel *et al.* (1985), Kren *et al.* (1987), Rozman *et al.* (1989), and Lohmeyer *et al.* (1990).

New alkaloids, feeding experiments for changing the alkaloid spectra: Flieger *et al.* (1989), and Udvardy-Nagy *et al.* (1981).

Clavines and simple lysergic acid derivatives are obviously easier to manufacture than peptide-type alkaloids. But the latter group is the most important one in the medical use. The development of strains able to produce these alkaloids was the main problem for many groups in the world not only in the past, but at present and in future also. Some of new scientific methods and techniques will affect these researches. There is for instance, the protoplasts technique firstly used for Claviceps by Spalla *et al.* (1976), simultaneoulsy by Stahl *et al.* (1977), and later by Robbers *et al.* (1979). Floss *et al.* (1979), Spalla & Marnati (1979), Keller *et al.* (1980,1981) and Schumann *et al.* (1987). The Italian group has also demonstrated the fusion technique starting from auxotrophic strains of *C. purpurea* (Spalla & Marnati, 1979).

Another possibility is the feeding of cultures (normal strains or mutants) with precursors or their analogues, etc. By using this method Beacco *et al.* (1978a, b) had prepared some unusual and new alkaloid analogues. Kobel & Sanglier (1979) had influenced the alkaloid spectrum of a strain by feeding of valine, leucine or isoleucine which are components of the peptide moiety of the ergotoxin alkaloids ergocornine, a-ergocryptine or b-ergocryptine.

Floss (1976) has estimated the current annual world production of ergot alkaloids at 4000 kg of peptide alkaloids and in excess of 12000 kg of lysergic acid. The price of lysergic acid is between 3000 and 4000 $/kg. These demands are not likely to be lesser in future. Therefore, many efforts are needed to make available the increasing requirement for ergot alkaloids above all of peptidetype alkaloids and partial synthetic derivatives.

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*Production of Rye Ergot in India

R.N. Thakur

Regional Research Laboratory, Jammu - Tawi

Introduction

THE word ergot is derived from the French origin Argot which means the fur of the bird. It is a common name used for the fungus *Claviceps* species which is produced as a result of infection of the fungus on flowers of rye. The several species of the genus *Claviceps* in fact the other members of the grass family, wheat, rye, oats and barley are the most important bread cereals cultivated on an extensive scale in most of the countries like Russia, U.S.A., Switzerland, France, Austria, Germany, Italy, U.K., Holland, Poland, Australia, Hungary, Bulgaria, Spain, Czechoslovakia and other European countries. During certain years due to the favourable weather and climatic conditions, ergot infestation used to become an epidemic, producing considerably large quantities of ergot sclerotia as a result of natural infection. In the early stages these countries took the initiative for separation and collection of these sclerotial bodies from the grains and the sclerotia thus, obtained were marketed to the pharmaceutical industries within their own countries and then abroad. Actually they had the advantage of holding the monopoly of the trade of the entire world. India, being one country where rye is not a commonly grown crop, had to completely depend upon other countries for the supply of ergot and ergot salts. It is for several years that entire world's demand of ergot was met by the natural collection of sclerotia from the diseased cereal crops. Due to enormous progress made in the advancement of agricultural sciences, especially in the field of studies of fungicides, the production and supply of agricultural chemicals for

*Revised and updated

plant protections had increased the ability of farmers to protect their crops effectively against commonly occurring dangerous diseases like ergot which used to result in heavy crop losses as also in mass poisoning of the large number of human and livestock population by ergot infection. This type of effective control measures enforced the control of the plant diseases in the said countries resulting in very little ergot information and medernisation of ergot under natural conditions. This created a worldwide shortage of this important drug. Therefore, most of the countries were deprived of their steady supply of this crude drug. Thus, these countries were forced to start in taking up artificial cultivation of this drug to meet the demand of the pharmaceutical industries within their own countries. India is one among the pioneer countries recorded for the artificial cultivation of rye ergot.

In India, till recently rye remained as an experimental crop in some of the livestock farms as a sort of green fodder. Therefore, the procurement of ergot of rye in large scale was only possible by artificial inoculation of rye crop and there was no chance at all to get ergot from natural infection. The entire demand of ergot sclerotia and its salts were being imported from the European countries. However, during the World War II, the entire supply of ergot and ergot preparations from other countries was stopped. Thus, the shortage of this essential drug made it necessary for the British Medical Council to initiate cultivation in several of their occupied colonies. An appeal was sent to all the British colonies to initiate artificial ergot production. India was one among them. The Agricultural Department of the then Madras Residency (Present Tamil Nadu) was among the first to start a project for ergot production with the financial help from the then Imperial Council of Agricultural Research (Present ICAR) in Nilgiris.

Thomas and Ramakrishnan (1942), Mundarajan *et al.*, (1950) carried out their extensive studies for suitable rye varieties, selection of strain of ergot fungus, sowing date in relation to yield of ergot, effect of altitude on flowering rye and manurial trial including the storage of ergot. The strain of ergot fungus they were using was of the ergotoxin group for which there was not much demand from the pharmaceutical industries during that period. Although they had all the technical know-how and suitable rye variety and a strain of ergot fungus owned by them was not able to initiate infection fully which resulted in low yield of ergot. Therefore, the attempts made for commercial cultivation under this project was found not feasible and the project was stopped. Similar project was also formulated to cultivate the rye at Rango Hills and produce artificially the rye ergot financed by the ICAR. Saha and Battacharjee (1945), Biswas (1959), have also conducted almost similar experiments like introduction of rye varieties, sowing and flowering date of rye for different altitudes etc. But the strain of ergot fungus used by them was of the same ergotoxin group and the method of inoculation followed by them was the spray inoculation method. Here again the yield obtained by them was low and was not suitable for the commercial cultivation.

In the meantime, the general awareness of public in India increased and drug consciousness has also increased proportionately, resulting in more demand for drugs in general and also for the ergot and its preparations in particular to meet the increasing demand for the crude ergot, our country started importing ergot and its preparations from various sources by spending valuable foreign exchange.

Table 1

Year	Name of the countries from which imports were made	Value of imports in rupees
1969 - 70	Germany	4, 72, 976
1970 - 71	U.K., Switzerland Bulgaria, Spain	9, 26, 500
1971 - 72	Hungary, Netherland	7, 72, 046
1972 - 73	Newzealand	8, 32, 623
1973 - 74	-	36, 94, 225
1974 - 75	-	51, 16, 892
1977 - 78	-	Data not available

The values given in Table 1 clearly indicate that the import of ergot and ergot preparations are increasing every year.

At this stage (1958-59), project was started in Regional Research Laboratory, Jammu to find out the possibility of ergot cultivation under the climatiic conditions or Jammu and Kashmir. Various trials conducted by earlier workers indicated that it is possible to produce pharmaceutical grade of ergot in Jammu and Kashmir, The strain of ergotoxin producing ergot was used and the same old spray method of inoculation was adopted (Gandotra and Ganguly, 1962). However, later (1964-65), attempts were made to acquire different strains of ergot cultures from various sources from different countries and grouped them into three distinct Chemical races producing ergotamine, ergometrine and ergotoxin (Singh, 1967 and Sastry *et al.*, 1970). During 1964, a project was formulated with specific objective as follow:

1. Selection and improvement of the strains of the ergot fungus.
2. Introduction and acclamatization of suitable varities of rye for commercial cultivation.
3. Improvement and standarisation of techniques for the commercial cultivation of rye ergot.
4. To meet the requirement of the country for ergot alkaloids.
5. To save the expenditure on foreign exchange spent in purchasing the drug and to earn the foreign exchange by the sale of ergot produced over and above requirement of the country.

Selection and Improvement of Strains of Ergot Fungus

The work carried out by the earlier workers in Tamil Nadu, West Bengal, Regional Research Laboratory, Jammu and Regional Research Laboratory, Jorhat (Assam) was entirely based on race of ergot fungus which produced sclerotia containing predominantely ergotoxin. The inoculation techniques followed by all these groups of workers was the spray method inoculation at the time of flowering of the rye crop. There was no market for ergotoxin strain nor the method of inoculation for the production of ergot was suitable. In this way, it used to result in the low yield of the ergot production in the country. The low yield factor became difficult for the commercial exploitation of the ergot production in the country. However, during 1964, a large number of cultures of ergot fungi obtained from various countries were classified into three distinct groups depending upon the type of ergot alkaloid produced by them. They were ergotimine, ergomatrine and ergotoxin. These strains were tested for their performance under agroclimatic conditions of both Jammu and Kashmir region (tropical and temperate) and some of them have been found to be suitable for the further use (Husain and Singh). Among the large number of cultures received from various sources, only ten of them originally having the desired alkaloid production capacity were selected. Six were of ergotamine type having the capacity to produce ergotamine 0.21 to 0.35% of total alkaloid, three of ergotoxine (0.16 to 0.30%) and only one of ergometrine type which yielded total alkaloid of 0.27%. The selected strains were employed to improve the alkaloid content by screening the single sclerotium analysis method every year. Finally, the selection and improvement programme was intensified for ergotamine strains production only and the total alkaloid content of the ergot sclerotia produced by some of the selected strains during 1967-78 are given in Table 2.

The best selected strain containing predominantly ergotamine has been used for the large scale production of ergot in collaboration with CIMAP farms and the approximate yield of ergot (sclerotia is given in Table 3).

Table 2 — Average alkaloid content of the ergot from selected strains

Strain	Percentage of total Alkaloids		
	1975-76	1976-77	1977-78
A	0.56	0.51	0.50
B	0.57	0.58	0.60
C	0.55	0.64	0.65
D	0.58	0.60	0.62
E	0.62	0.63	0.65
F	0.61	0.66	0.65
G	0.63	0.68	0.67
H	-	0.32	0.45

Table 3 — Yield of ergot from the improved strain (R-56)

Year	Approximate area of hectare	Approximate yield in quintals
1967-68	82	9.87
1968-69	49	7.58
1969-70	56	17.13
1970-71	74	37.57
1971-72	74	39.57
1972-73	74	36.90
1973-74	117	102.80
1974-75	180	145.00
1975-76	123	106.00
1976-77	160	160.00
1977-78	120	100.00

The yield obtained in the year 1976-77 indicates that there is much improvement in the selected strain in comparison to previous years with regard to quantity (Table 3) and also quality (Table 2) of ergot production (Husain, Singh and Thakur, 1976).

Selection of Suitable Rye Variety for the Commercial Ergot Production

Work on introduction and acclamatization of suitable rye variety was started in 1945 when the project for artificial ergot production was initiated in Nilgiris, Rango hills and Regional Research Laboratory, Jammu. But no rye variety has so far been able to replace the originally obtained variety from Italy and acclamatized by the Tamil Nadu agriculture department for the artificial ergot production. However, Dr. C.K. Atal collected 575 individual rye cultivars from all over the world. They were screened for their suitability in Jammu and Kashmir conditions. Only three cultivars of diploid rye were found to be suitable for both the regions but in the further trials they could not be found better than original one.

Selection of Site

Selection of site for growing rye for the production of ergot is one of the important factors. A natural valley at an altitude of above 2000 meters above sea level with moderate snow fall and humidity varying from 60-90 per cent would be an ideal place. The land should preferably be uniformly level, moderately fertile and located in a fairly humid environment. The most suitable is a valley like Kashmir or plane land surrounded by a forest located near a river.

Soil and Water Requirement

The soil for wheat and barley is exactly the same for rye but loamy soil is the best suited for a very good crop with proper application of fertilizers and irrigation. In comparison to the other cereals rye requires moderately larger quantity of water to maintain moderate humidity for the development of ergot sclerotia. It is always better to maintain moist soil during the development of sclerotia from inoculation time to maturation of ergot in the field.

Sowing of Rye

Sowing of *Crotalaria juncea* L. for green manuring is good for improving the soil conditions in many ways also resutls in good yield of ergot. The sunhemp should be ploughed before flowering and it improves the tilth of the soil. In case, there is no green manuring, 4 to 6 tonnes per hectare of FYM is to be applied. Sowing of rye may very from place to place for which the rye crop is raised. But if it is for ergot cultivation, it is advisable to sow the seeds with the help of a seed drill in rows to obtain uniform crop Sowing on ridges inside the beds under intensive cultivation has been found to be very good for irrigation, manuring and also to be very good for irrigation, manuring and also to get better crop and higher yield of ergot sclerotia when compared to the crop sown in furrow. About 70-80 kg. of rye seeds are sufficient to sow in one hectare of land in rows 20 cm apart. Sowing of rye varies due to factors like climate, topography and elevation of the place. Last week of September middle of October is the optimum time of sowing to get good yield of ergot in Jammu region. In the Kashmir valley September to middle November has been found to be suitable period for raising of rye crop for the ergot production. Under Jammu and Kashmir valley conditions, 80 kg ammonium sulphate, 30 kg murate of potash and 25 kg super phosphate per hectare has been found to be the optimum requirement.

Preparation of the Inoculum

The success of the ergot cultivation mainly depends upon the selection of proper strains of the ergot fungus, selection of suitable medium for the production of large scale inoculum and optimum temperature for the multiplication of inoculum. The selected strains should be able to produce enough sclerotia with desired percentage of required alkaloid. Virulence of any pathogen is gradually lost if the pathogen is subjected to continuous sub culturing on the artificial medium without passing the strain through its host every year. The general method for reisolation of a new culture is to select carefully the well developed and fully matured sclerotia directly from the field or from the bulk after harvest. Generally one sclerotium out of one thousand sclerotia is selected and in this way, ten higher alkaloid containing sclerotia are selected on the basis of single sclerotium analysis method. In this way, ten sclerotial bits after selection ˎ n the basis of higher alkaloid content are used to

isolate the culture and these are mixed together for the preparation of bulk inoculum.

Selection of Suitable Container

In the beginning there was no suitable container for the preparation of bulk inoculum for the production of ergot in a commercial scale. 250 ml to 1 litre capacity of flasks were tried but found difficult to check the breakage due to delicate neck and basal potions. This being costly affair gradually the flasks were replaced with ordinary milk bottles of 500 ml. capacity which is hard in handling and cheap in cost and easily available. Now the milk bottles of 500 ml. have become the common and most suitable container for the large scale inoculum production for the commercial ergot production.

Medium for the Ergot Inoculum Seed Medium

The medium forms one of the most important factor on which the ergot fungus has to grow and sporulate profusely. Since the experiments conducted in this laboratory revealed that wheat and rye are equally good as the medium but wheat seeds has been found better than rye as the fungus grows and multiplies rapidly surrounding the individual seeds. However, the time taken for the preparation of one set of ergot inoculum is 4-5 weeks from the date of inoculation and the inoculated bottles are kept at 20-22°C ± 2 and this is ready for inoculation. Fully grown bottles of inoculum can be stored upto another 4-5 weeks at 10-20°C. Generally 30-40 days old inoculum is used for the inoculation of rye crop.

Synthetic Medium

The medium prepared from wheat seeds is of good quality for the both purposes as to inoculate the rye ear heads as well as to store for sometime without any deterioration or lysis of the culture. Synthetic liquid medium takes short time of 10 -12 days but it takes less infection and cannot be stored for a long time. It is comparatively easy but requires immediate inoculation.

Quality of the Inoculum

Inoculum prepared by any method should atleast contain desired number of viable spores roughly estimated to be 3000-4000 spores/mm of the inoculum suspension (Sastry *et al.*, 1970). But the synthetic medium used for the inoculum preparation does not contain so much spores as desired for the inoculation of rye ear head.

Stage of Rye Species for the Inoculation

Inoculation of rye ear heads was usually done by earlier workers by spray method of inoculation at the time of the maximum flowering of rye spikes but it was defective method. For the inoculation by needle board puncture method, the majority of the rye spikes in the field should be out from the boot leaf and the remaining in the bootleaf. By needle board puncture method the inoculum is injected into the closed glumes which are yet to open. There will be sufficient moisture inside the glumes to make the inoculated ergot spores to come down to the basal region of the florets where exactly the ovarian base is situated. The spores get sufficient moisture nutrients inside the closed glumes and that is appropriate place for the germination and establishment of ergot fungus before rye flowers get fertilized. Thus, the inoculation by needle board puncture method is naturally superior over the spray method of inoculation.

Development of Sclerotia

In general winter rye varieties have been reported to be superior over the spring varieties for the ergot production. However, the tetraploids have been reported to be far more superior than the diploid strains because of their large spikes. In addition to large size of the spikes, the sclerotia produced by tetraploid rye varieties have been reported to be 2-3 times bigger, heavier and higher alkaloid content when compared to ergot produced on diploid rye.

Number of Sclerotia on Individual Spike

Variation in alkaloid content has been observed in ergot sclerotia of the same strain of ergot fungus (Tyler, 1962 and Tanda, 1965) when they are produced in different numbers on individual spikes of rye. The experiment carried out in RRL reveal that an average total alkaloid content was about 0.45% when the number of ergot sclerotia produced on an individual spike remained within 1-3 in numbers and decreased to about 0.35% when the number increased to between 4-6. Further decrease in alkaloid content (0.30%) was obvious when the number of sclerotia produced on an individual spike exceeded more than 7.

Variation in the Alkaloid Content of Rye Ergot by the same Strain of Ergot Fungus

Variation in fungi is a common phenomenon and it is very common in the ergot fungus *(Claviceps purpurea)*. The results of the experiments carried out to study the probable number and the range of ergot variable percentage esclaration containing ergot alkaloid in general samples of sclerotia, produced by the same strain of the ergot fungus have been reported by Sastry *et al.*, (1977). A group of samples obtained from a selected strain of ergot fungus, which predominantly produced

ergotamine, were further selected at random from the ergot produced in 1975-76. The single sclerotial analysis was carried out for each bit of the ergot sclerotium according to the procedure specified under British Pharmacopoeia (1968). On the basis of the total alkaloid and ergotamine percentage obtained from the chemical analysis of each bit of the individual sclerotium, they were grouped into eight categories (Table 4).

Table 4 — Range in variation of total alkaloid content of the single ergot sclerotium produced by the same strain of the ergot fungus

Optical density	Total alkaloid per cent	Sample in each range out of one thousand	
		No. of samples	Percentage
0.4 - 0.60	0.320-0.480	75	7.5
0.61 - 0.80	0.481-0.640	73	7.3
0.81 - 1.00	0.641-0.800	188	18.8
1.01 - 1 20	0.801-0.960	195	19.5
1.21 - 1.40	0.961-1.120	341	34.1
1.41 - 1.60	1.121-1.280	83	8.3
1.61 - 1.80	1.281-1.440	44	4.4
1.81 - 2.00	1.441-1.600	1	0.1

Similar variation in the percentage of ergotamine was also obtained by calculating the individual alkaloid. The highest percentage of ergotamine recorded from these samples was 70%.

Age of the ergot inoculum in relation to the yield of ergot and alkaloid content:

The existence of variability in alkaloid content of the ergot produced on different strains under various conditions has been reported by many workers. Survey of literature indicates that very little information is known about the age of inoculum and its effects on the infection of ear heads and yield of ergot and alkaloid content. In this connection, an experiment was conducted with various age group of ergot cultures which were maintained from 1971-72 to 1975-76 in RRL, Jammu. All the optimum conditions for the preparation of inoculum, inoculation of rye ear heads by needle board puncture method were followed. Five ergot sclerotia in each treatment were analysed by British pharmacopoeial method. The data presented in Table 5 indicate that the yield of the ergot is universally proportional to the age of the culture and also obtained gradual decrease in the per cent of alkaloid content produced with regard to the age. The results clearly show that in general there is a gradual decrease in virulence and alkaloid producing capacity of the ergot culture isolated and maintained in artificial medium without passing them through their

Table 5 — Effect of age of ergot culture on the yield and alkaloid content

Culture used of the year	Yield of ergot (kg)			
	Per plot	Per hectare calculated	Total alkaloid	Ergotamine
1971-72	0.456	32	0.36	0.115
1972-73	0.534	106	0.39	0.145
1973-74	0.727	145	0.43	0.138
1974-75	1.017	202	0.45	0.175
1975-76	1.296	260	0.53	0.285

hosts. Therefore, the isolation of fresh culture every year is the only best way to maintain proper virulence and alkaloid content for the production of ergot sclerotia on large scale.

Preparation of Inoculum for the Large Scale Ergot Production

Preparation of the inoculum is as important as any other operation in production of ergot sclerotia on commercial scale. The approximate cost of production of the inoculum in bulk on sterile rye/wheat grains is Rs. 5/= per bottle. Ordinary milk bottles of 500 ml capacity having 150 ml. of wheat grains and 150 ml of water are the best suitable containers. It is also suitable for the purpose of autoclaving, handling, packing and transporting.

Standardisation of the Inoculum

Suitable inoculum for the correct inoculation of the rye spikes always depends upon the optimum concentration of spores in the fresh inoculum. It is the important factor for the commercial production of ergot inoculum, 40-50 days old culture on wheat medium forms the best inoculum with all the desired characters. The general procedure is to take the whole content of the culture from bottles and mix it with 4.5 litres of water and separate the grains. This concentration is the ideal for the rye inoculation.

The rye spikes should be inoculated twice, one at stage of 50% emergence at spikes and the second inoculation at the stage of 80-90% of emergence by the needle board puncture method. The prepared inoculum should be used on the same day if not possible then it should be used within 2-3 days but should be stored below 10°C.

Stage of Rye Spikes

Healthy crop of rye is the important factor which determines the economy of the commercial production of ergot sclerotia. In addition to the primary factors men-

tioned for the inoculum and its storage, climatic conditions at the time of the development of ergot sclerotia, methods of inoculation of the spikes and stage of rye at the time of inoculation are also the main factors which certainly play an important role in determination of ergot yield. It was found by the experiments conducted at RRL, Jammu that the inoculations made at the time when one third or more than half of the top portion of rye spikes was out from the boot leaf gave the maximum yield of sclerotia.

Method of Inoculation

There were several method followed by different workers when the ergot cultivation was initiated. For getting the maximum infection and yield of ergot, the needle board and rubber pad puncture method was found to be the suitable for the large scale production of ergot. In this method the sponge rubber board is dipped in the inoculum suspension and the spikes of the rye plants are caught in between the needle board and the rubber covered board held in each hand exactly opposite to each other and pressed gently and uniformly as for as possible so as not to damage the spikes. Tnis method is superior over the spray method and can obtain 2-3 times more yield of ergot than spray method. It was also observed that two times inoculation of rye of proper stage is definitely better than one time or more than two times.

Harvesting of Ergot Sclerotia

Generally it has been suggested that the ergot sclerotia should be collected after six to eight weeks of inoculation But the experimental records show that ergot sclerotia should be collected when the rye is almost ready to harvest. However, with all the care, well developed sclerotia are generally ready for picking somewhat earlier than rye harvest. Because they are liable to get dislodged from the spikes with slight jerk due to wind or rains. It is advisable to pick up two times (1) first, when majority of sclerotia are fully matured and second time when the rye is ready to be harvested. If the sclerotia are not picked up timely they fall down on the ground.

The sclerotia, which remain in standing water for more than 12 hrs completely become useless for the pharmaceutical purposes. One man can collect 1-3 kg of ergot in about 6-7 hours. The yield of ergot is highly variable under Indian conditions.

Drying of Ergot

Picking of ergot generally starts in the month of April under Jammu conditions. Whereas in Kashmir valley it starts in July-August. Drying is carried out under shade in open air. In beginning turning is required as fresh sclerotia contain 10-20 per cent moisture in fresh collection. The moisture content of ergot should not be more than 5-6% at the time of storage.

Cost of Production of Ergot

The cost of ergot production depends on yield of ergot per unit area. The approximate cost of production is Rs. 24.55 per kg. This can be reduced to Rs. 20 per kg, if the yield of ergot is more than 100 kg/hectare as it comes in Kashmir Valley.

Spread of Ergot Sclerotia to other Cereal Crops

It is a general feeling that this ergot fungus (*C. purpurea*) can infect the other crops. Generally infection does not take in wheat, barley and oat. Only a few spikes get infection. Different types of wheat and barley varieties were inoculated artificially and the result showed that without punching the spikes of these crops takes infection in a few spikes which can be avoided by keeping off the other crops for the minimum 100 yards.

Survival of Sclerotia

An experiment was carried out to find out the viability of the ergot fungus under field conditions of Jammu region. The sclerotia obtained from fresh crop were spread on the surface of the field in different places where the rye crop was grown for the ergot cultivation. The sclerotia were buried on different places at the depth of 7.5 and 15 cm deep in same field also kept on the surface of the field. The samples collected and isolated on PDA after the interval of one month. The samples obtained upto end of June were viable but the samples of July could not germinate. Sclerotia in Jammu region could not survie due to temperature and humidity after July.

Present Status for the Rye Ergot Cultivation

Proper selection of location, suitable variety of rye and improved strain of *Claviceps purpurea* are the main points to be considered for the cultivation of rye ergot in the country. Agronomically suitable field, green manuring of sunhemp, timely application of proper combination of fertilizers; irrigation, hoeing, weeding are important for growing rye. Inoculum of desired strains of *Claviceps purpurea* for ergotamine, ergometrine and ergotoxin must be selected from fresh and viable sclerotia after analysis of the desired alkaloid contents. Pure culture of 40 days old in milk bottle of 500 ml capacity containing wheat grains 150 gms and water 150 ml is the most suitable for the inoculation by needle board puncture method. The milk bottles are hard for the sterilization, easy for inoculation and incubation for the growth and transportation. The suitable temperature is 22°C ± 2 during incubation period which is maintained for 40-45 days for the complete growth and sporulation. One milk bottle containing ergot culture and 4.5 litres of water of the optimum concentration of spores for the inoculation of rye ear heads. The emergence of rye ear heads must be 50-60% for the Ist inoculation and about 75-80% emergence of ears for the second inoculation after the interval of 10-15 days.

100-150 kg ergot are obtained in Kashmir valley. At present the places similar to Kashmir valley are being used for the cultivation of rye ergot by a sister Institute CIMAP, Lucknow all over the country for meeting the demand of the ergot alkaloids.

Acknowledgement

Author is highly thankful to Dr. R.S. Kapil, Director, Regional Research Laboratory, Jammu for his keen interest and encouragements for preparing this manuscript and thanks are also due to Dr. B.L. Kaul, Botanical Sciences, for this valuable suggestions.

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Production of ergot alkaloids by submerged fermentation

R.N.Thakur

Division of Botanical Sciences
Regional Research Laboratory
Canal Road, Jammu-180001

Introduction

ERGOT alkaloids have long history of toxic manifestations and medicinal uses to mankind. These tetracyclic indole derivatives are primarily found in the sclerotia of various species of *Claviceps* which parasitize a large number of species of family poaceae. Based on their chemical structures, the alkaloids of ergot can be divided into two classes: clavines and lysergic acid derivatives. Both contain thė tetracyclic ergoline ring system in which the substituent at C-8 has the oxidation state of methyl or hydroxymethyl group, and C-8 and C-9 may carry an additional oxygen. The lysergic acid derivatives are of prime interest to pharmacologists due to their effects on uterine and vascular smooth muscles and sympathetic nervous system. Durıng the recent past, new aspects of the pharmacology of the ergot derivatives have been very interesting. Certain ergot derivatives act as inhibitors of prolactine secretion, produce stimulation of dopaminergic receptors and certain newer ergot derivatives have been implicated as potential therapeutic agents in diseases such as parkinsonian suppression of postpartum lactation, treatment of breast cancer and cancer of the prostrate. The clavines have until recently been of little importance from the medıcinal point of vıew. Some of them are, however, intermediate in the formation of lysergic acid derivatives. The first group, the amide derivatives of lysergic acid, shows the broad spectrum of pharmacological effects and that has led to the wide spread medicinal uses of ergot alkaloids and their semi-synthetic derivatives. The

current medicinal uses are — (1) Treatment of postpartum haemorrhage; (2) Migrainic headache; and (3) Psychotherapy. Some of the compounds prepared from clavine type of alkaloids were found to be prolactin inhibitor. One of the compounds prepared i.e. 6-methyl-8 ergolenylacetamide was very potent comparing favourably in activity to the best prolactin inhibitors reported to date. Mantle (1969) confirmed this antilactating effect of clavine type of alkaloid. Two groups of clavine alkaloids namely elymoclavine and agroclavine may be coupled with each other and each couple with dihydro-d-lysergic acid. This gives us an idea of the interrelationship of the clavine ergot alkaloids among themselves and with those of classic type. As for the structure, it was found that agroclavine was not derivative for lysergic acid. As it contains no carboxyl group, no double bond at position 9 and 10 and no peptide portion, it is just a new alkaloid — an ergotine-8- derivative. It has CH_3 at position 8 instead of a carboxyl group. Thus, we know that its structure is similar to though not the same as that of the lysergic acid portion of the ergot of rye alkaloids.

Sources of Ergot Alkaloids

Ergot alkaloids are obtained by the extraction of ergot sclerotia produced on different grasses of family poaceae under natural conditions. Secondly the ergot sclerotia are obtained from the artificial cultivation of ergot of rye. Thirdly by the exploitation of saprophytic culture of different species of *Claviceps* by using chemical strains of ergot fungi and lastly by partial or total synthesis of ergot alkaloids. The different group of alkaloids namely ergotamine, ergometrine, ergokriptine, ergocristine, ergocarnine, simple lysergic acid derivatives, lysergic acid and phospalic acid are produced by various pharmaceutical companies

Though the exact statistics are not available from individual countries about the correct requirement of the ergot alkaloids and their derivatives, the total annual requirement of the entire world is approximately estimated at 4,000 kg of the peptide type and more than 12,000 kg of simple lysergic acid derivatives The approximate cost of the lysergic acid in the international market varies from Rs. 25,000 to 32,000 per kg.

Factors affecting the Production of Ergot Alkaloids under Submerged Culture Condition

Production of ergot sclerotia by parasitic method depends upon mainly climatic conditions which may be favourable or may not be every year during crop season. Secondly growing population requires sufficient land for the production of food grains to meet the requirement of the country. The only alternative is to produce the ergot alkaloids by submerged culture conditions. Two strains numbering A6 and B14 of *Claviceps paspali* Stevens and Hall were taken for LAD under submerged culture conditions. The strain P921 of *Claviceps* spp. was employed for the production of clavine type of alkaloids.

Table 1: Selection of isolates for the production of LAD after the treatment of γ-rays

S.No.	Isolate No.	Doses of γ-rays in Krads	Alkaloid content in mg/L	Incubation period
1.	A-1	50"	669.6	10 days
2.	A-2	" "	734.4	"
3.	A-5	" "	669.6	"
4.	A-96	" "	669.6	"
5.	A-1-13	" "	554.0	"
6.	A-1-4	100"	712.6	"
7.	A-1-19	" "	756.0	"
8.	A-20-1	" "	885.6	"
9.	A-22-7	" "	842.4	"
10.	B-8-1	50 "	1026.0	"
11.	B-8-2	" "	1080.0	"
12.	B-3-11	" "	691.1	"
13.	B-4-3	" "	669.6	"
14.	B-88-3	" "	669.6	"
15.	B-123-1	" "	779.6	"
16.	B-123-2	" "	680.4	"
17.	B-123-8	" "	723.6	"
18.	B-123-3-1	100"	1188.0	"
19.	B-127-1	" "	771.2	"
20.	B-127-2	" "	864.0	"
21.	B-127-8	" "	777.6	"
22.	B-127-2-1	" "	734.4	"
23.	B-127-7-11	" "	907.2	"
24.	B-132-17	" "	896.0	"
25.	B-132-18	" "	756.0	"

Table 2 : Effect of age of inoculum on the production of alkaloids

Type of inoculum	Incubation period for the production of alkaloids content in mg/L.		
	6 days	8 days	10 days
Fresh inoculum	594.0	831.6	896.4
7 days old	562.0	734.4	658.8
14 days old	615.0	461.0	378.0

Maintenance of Cultures

The stock cultures of all the three strains namely A6, B 14 and P921 were maintained on potato dextrose agar slants at $5^{\circ}C\pm1$ in screw cap culture tubes. Active cultures were employed in the experimental trials. First they were transferred on PDA poured plates and then after 7 days the cultures were sub-cultured on PDA slants. Ten days old culture tubes were used in each experiment. Seed and production media were employed for both types of alkaloids with different concentrations of ingredients (Table 3). The factors like sources of carbon, nitrogen, phosphate, sulphate and trace elements, were optimised. The other factors affecting the yield of ergot alkaloids like improvement of strains, stage of seed culture, size of inoculum, storage of inoculum etc. were studied to find out the optimum conditions other than said factors, pure chemicals of A R grade and double distilled water was employed in seed and production media.

Fermentation Procedure

Commonly three stage fermentation procedure was followed throughout the investigation. Ten days old, one square centimeter of the mycelial bit was taken for the Ist stage seed culture. It was mixed with nutrient liquid medium and homogenized for 20 seconds in an omnimix; After homogenization the homoginate was taken back into the same sterile conical flask of 500 ml. It was incubated for 6-8 days on rotary shaker operating at 230 rpm. The temperature of the incubation room was maintained at $24^{\circ}C\pm1$, when the full growth was visible, the entire content of the mycelial growth of stage one, seed culture flask was again homogenized for 10-15 seconds for stage second. Ten ml of homogenized mycelial suspension was added to 500 ml flask containing 100 ml of seed medium for the stage second (Table 4) After inoculation, the flasks were incubated for stage second under similar condition mentioned for stage first for 72 hrs. To begin with stage three, ten ml of mycelial suspension from stage second seed culture was transferred into 500 ml of flasks containing 100 ml of the production medium. The production medium was containing various types of sucrose and mannitol, nitrogen sources, phosphate, sulphate and trace elements (Copper sulphate, zinc sulphate, iron sulphate and manganese sulphate). The flasks containing production medium were incubated for 10-12 days under similar conditions after inoculation with 10 per cent of inoculum of 2nd stage seed culture. All the experiments were repeated three times in three replications.

Sampling and Analysis Procedure

About 10 ml of fermented broth was taken out each time from each treatment under aseptic conditions with the help of separate sterile pipette for the analysis of alkaloid content. The sampling was made at the interval of 6,8,10 and 12 days of inoculation of production media. The mycelia were separated from the broth of each sample by suction filtration through double layer of Whatman No. 1 filter paper disc placed in Buckner funnel. The mycelial free broth thus obtained was used individually for recording *p*H and optical density. The *p*H of each individual sample was recorded

Table 3: Medium for the production of ergot alkaloids in submerged culture

Ingredients	Seed Medium	Production Medium
Succinic acid	10 gm	30 gm
Mannitol	40 gm	50 gm
KH_2PO_4.	0.1	0.1
$MgSO_4$	0.6	0.6
$NaNO_3$	0.1	0.1
$Ca(NO_3)_2$	0.2	0.3
$FeSO_4$	0.01	0.01
$ZnSO_4$	0.01	0.01
$MnSO_4$	0.08	0.08
$CuSO_4$	0.01	0.01

Table 4 : Selection of isolates for the production of Clavine type of alkaloids

Isolates	Yield in mg/L	Incubation period in days
Mother culture 921	812	10
2A	712	"
2C	1052	"
4	698	"
12	756	"
43	797	"
48	663	"

Table 5 · Effect of mannitol and sucrose in the yield of clavine type of alkaloids producing isolates.

Isolates	Yeild in mg/L		Incubation period in days
	Manntol	Sucrose AR	
921	856	1080	10
2A	1080	1620	"
2C	1296	1620	"
4	872	1196	"
12	929	1026	"
43	1090	1572	"
48	907	1404	"

separately and the quantity of lysergic acid derivatives (LAD) present in the filtrate was estimated by recording colorimetric readings obtained at 590 nm in Elico spectro Model-CL-23. The total alkaloid present was calculated from the calibration curve prepared from an authentic sample of ergotamine maliate. The result are expressed in mg/L. The colour development was done with the help of van urk reagent (para dimethyl amino benzaldehyde) in 65 per cent sulphuric acid of A R grade. All the glass wares and the appliances used in the experiments were washed thoroughly in the detergent solution and cleaned five times with hot water. They were again washed with HCl and distilled water and finally with double distilled water before drying in oven.

Improvement of Strains

Three strains numbering A6, B14 and P921 were selected for the improvement of their alkaloid contents. They were given the exposure of 12 doses of γ-rays. The treated tubes (5 tubes in each case) were mixed with distilled sterile water and homogenized them in sterile container for 30 seconds. The mycelial suspension were diluted upto the extent to get the single colony from one mycelial bit. They were transferred into PDA tubes and used to test them for the production of alkaloid content In each case 100 colonies were screened out of twelve doses and the fungal cultures of both strains survived upto 100 Krads. Only two doses 50 and 100 Krads were found to be effective and yielded the higher alkaloid content of 25 isolates than the original mother strains A6 and B14 (Table 1). Out of 25 isolates only one isolate numbering B- 123-3-1 yielded the maximum of 1188 mg/L of LAD. It was taken for the further optimization of physical and chemical parameters for the improvement of the yield of alkaloid content (LAD).

Factors affecting the yield of Ergot Alkaloids

Age of the Initial Culture

Age of the culture for the production of ergot alkaloids plays an important part in the study of the physical and chemical factors under sub-merged culture conditions A mycelial bit of fungal culture should be taken from a fully grown young active colony. Five different age group cultures selected were 10, 15, 20, 25 and 30 days old grown at 25°C±1 on PDA agar slants.The alkaloid content obtained revealed that the initial cultures of the age group of 10-15 days old were found to be better than the older colonies for both type of cultures producing LAD and clavine alkaloids. In ergot alkaloid fermentation, generally three stage method is followed by majority of workers (Abe *et al.*, 1967, Bhat *et al.*,1976 and Kellcher, 1970). Stage first and second may vary and depends upon the growth of the particular fungal culture but the production period is generally completed within 10-12 days of incubation. Under the trial of concentration of inoculum for the production medium, five concentrations of 5, 10, 15, 20 and 25 per cent of the total volume of the production medium were used. The results obtained indicated that increase or

decrease in the concentration of inoculum beyond ten per cent could not improve the yield of alkaloid content. This concentration of inoculum was equally good for the clavine type of alkaloids.

Duration of Fermentation in Production Medium

To find out the duration of incubation period for the production of ergot alkaloids under submerged culture condition, it is an important factor to decide the duration period for the maximum yield of the alkaloids. Four duration periods were taken into consideration. The samples were taken aseptically at the end of 6, 8, 10, 12 and 14 days after inoculation of production medium. It was observed that the maximum yield of LAD and clavine alkaloid was found on 10th day. Shorter and longer period of production could not increase the yield of alkaloid content.

Storage of Inoculum

To find out the effect of storage of inoculum three durations of fresh, one and two weeks were undertaken. The pure inoculum of second stage seed culture was stored at $5^{\circ}C\pm1$. The production medium was inoculated with 10 per cent of each group of stored inoculum and allowed them for the fermentation for 10 days at $25^{\circ}C\pm1$ at 230 rpm of rotary shaker. All the four samples were analysed and found that freshly prepared inoculum was better for the production of biomass as well as for the alkaloids content in comparison to older and stored inoculum. The stored inoculum was found to decrease the yield of ergot alkaloids of both types. The results are presented in Table 2. The results show that the fresh inoculum used for the inoculation of production medium increases yield upto 10 days and stored inoculum used were observed to decrease the yield. This study reveals that fresh inoculum for the inoculation of production medium is better Older inoculum starts lysis earlier and declines the yield of the alkaloids also.

Buffering of the seed and production media with sodium dihydrogen phosphate does not increase the yield of alkaloid content of any one of alkaloids under test. To check the foaming in production period, three concentrations of groundnut and sunflower oils were employed. There was no change in the yield of any one of the alkaloids. In the separate trials, four concentrations of potassium dihydrogen phosphate were used separately for each of two alkaloids by keeping the other parameters the same. The results showed that 1 g/L was more suitable concentration than below or above concentrations for the production of both types of alkaloids. In the application of magnesium sulphate, ten concentrations were employed but the data indicated that 0.6g/L was better than any increase or decrease for production of clavine type of alkaloid (Table 11). The same thing was observed for the LAD production. Six concentrations of calcium nitrate were employed in the production as well as seed media for the improvement of the yield of alkaloid contents. The data presented in (Table 12) indicated that 0.2 to 0.3g/L was more effective than any other concentration under test. Similar finding was recorded for the production of LAD under submerged culture conditions.

Table 6 : Effect of automization of inoculum before inoculation of production medium

Isolates	Yeild in mg/L		
	Homogenized	Nonhomogenized	Incubation in days
2A	1512	1080	10
2C	1620	1080	"
4	995	856	"
12	1296	993	"
43	1512	1088	"
48	1512	929	"

Table 7 : Effect of different grade of mannitol on the yield of alkaloids (LAD)

Grades	Yeild in mg/L	Incubation period in days
B.P.Grade	824.8	10
B.P.Grade further purified	894.0	10
Extra pure	972.0	10
Extra pure further purified	1025.0	10
IDPL	1025.0	10
IDPL further purified	1496 0	10

Table 8 : Effect of different carbon sources on the yield of alkaloids (LAD)

Sources of carbon	Yeild in mg/L	Incubation period in days
Mannitol B.P	712.8	10
Mannitol B.P. purified	734.4	"
Mannitol Extra pure	734.4	"
Mannitol Extra pure purified	771.2	"
IDPL	669.0	"
IDPL purified	972.0	"
Glucose	518.4	"
Galactose	529.4	"
Lactose	302.4	"
Sucrose	1101.8	"

Table 9: Effect of different conc. of sucrose for the production of clavine alkaloid

Conc of Sugar in per cent	Yield of alkaloid in mg\L	Incubation period in days
Mannitol	1583.2	10
Sucrose 1	1771.2	"
Sucrose 2.5	1712.8	"
Sucrose 5.0	1742.8	"
Sucrose 7 5	1712.8	"
Sucrose 10.0	1669.6	"
Sucrose 12.5	1669.6	"
Sucrose 15.0	1669.6	"
Common sugar 5	1712.8	"
Common sugar 7.5	1734.4	"

Table 10: Effect of acids of carboxylic group on the yield of clavine alkaloid

Acid 3 per cent	Yeild in mg/L	Incubation in days
Succinic acid	986.6	10
Citric acid	183.6	"
Acetic acid	54.0	"
Fumaric acid	172.8	"
Maleic acid	334.4	"
Oxalic acid	712.8	"
Tartaric acid BDH	432.0	"
Tartaric acid IDPL	205.2	"
Benzoic acid	75.9	"
No acid	340.4	"

Table 11: Effect of magnesium sulphate on the yield of alkaloids (LAD).

Conc.in g/L	Yield mg/l	Incubation in days
0.2	1069.2	10
0.4	1512.0	"
0.6	1620.0	"
0 8	1512.0	"
1.0	1512.0	"
1.2	1512.0	"
1.4	1432.0	"
1.6	1296.0	"
1.8	1188.0	"
2.0	1080.0	"

Requirement of Carbon Sources

According to the literature available, it was observed that mannitol as a carbon source and succinic acid for organic nitrogen was recorded by Abe *et al*., 1967, Taber and Vining 1957, Kelleher, 1970 However, in the present study it was observed that there was a lot of variation in the yield by the use of different grade of mannitol and other sources of carbohydrates (Table 8) Mannitol of IDPL purified was found to be superior than other sources of mannitol for the production of LAD (Table 7). But in the case of clavine alkaloids sucrose 5 per cent AR grade was found to be better than any other source of carbohydrate (Table 9). This indicated that sources of carbohydrates are not the same for all types of strains of alkaloid producing species of *Claviceps*. It was also found that addition of tryptophane does not play any significant role for the production of any type of ergot alkaloids under submerged culture conditions. Taking into consideration all the factors of physical and nutritional requirements for the production of ergot alkaloids under submerged culture conditions, it is also one of the important factors to homogenize the first and second stages of seed cultures before inoculating the production medium. The results indicated that the homogenized culture increased the yield of alkaloids of all the six isolates of *Claviceps* spp. Under test (Table 6) However, some of the factors directly affecting the yield of clavine type of alkaloids were optimized A strain P921 of *Claviceps* spp. was treated with 12 doses of γ - rays None of the culture tubes under test could survive beyond 100 Krads One hundred colonies from each of two doses (50 and 100 Krads) were tested for the production of clavine alkaloids. An isolate no 2c producing the maximum yield of this alkaloid was further selected for the optimisation of some factors affecting the yield of clavine alkaloids (Table 4). The fungal colonies grew comparatively better at 25 C$\pm$1 and *p*H 5.2. For the optimisation of carbon sources sucrose AR and mannitol were tried and results obtained have shown that sucrose AR was better than mannitol for all the isolates under test (Table 5). Similarly for organic source of nitrogen, succinic acid 30g/L was found better than any other concentrations for this alkaloid. For the optimisation of source and concentration of phosphate, potassium dihydrogen phosphate 100 mg/L was determined to be the better source than any other concentration. However, dipotassium monohydrogen phosphate (K_2HPO_4) could not yield the higher alkaloid with its four contractions. It is well known fact that production of any pure type of ergot alkaloid is very sensitive and particularly chemical have significant role in the production under submerged culture conditions. The maximum yield was obtained by the use of purified mannitol IDPL grade which was 1101 mg/L in comparison to nine sources of carbohydrates (Table 8) It was further confirmed by a separate trial of different concentrations of sucrose that 5% sucrose of AR grade was superior than any grade of sucorse (Table 9). However, different concentrations of inorganic nitrogen of calcium nitrate and sodium nitrate were tried and the results obtained indicated that 0.2 to 0.3% of calcium nitrate was better than any other source and concentration

of nitrate (Table 12). A trial with four trace elements namely ferrous sulphate, zinc sulphate, copper sulphate and manganese sulphate was conducted and the results obtained indicated that deletion of any one of the four trace elements adversely affects the yield of LAD. More over, clavine types require only two *viz*. Ferrous sulphate and Manganese sulphate and the remaining two were found to be inhibitor for the production of clavine type under submerged culture conditions (Table 13). Incubation period for the stationary culture was attempted and the result showed that the clavine alkaloids increased the yield upto 42 days and started to decline after 48 days (Table 14). However, it does not appear to be economical as it takes long time of incubation to complete the circle of clavine type alkaloids production. Secondly it requires space under aseptic condition for the growth and production of clavine alkaloids. In another set of experiment, seven carboxylic group of acids were tried and the results showed that succinic acid was the better source of organic nitrogen. The optimum concentration of succinic acid used for the production of LAD was again implemented for the production of clavine type of alkaloids in the same concentration. All the acids under test were found to be inferior for the clavine alkaloid production (Table 10) in comparison to succinic acid.

Table 12: Effect of calcium nitrate on the yield of clavine alkaloid

Conc. mg/L	Yield in mg/L	Incubation in days
0.0	1058.4	10
0 1	1512.0	"
0.2	1620.0	"
0 3	1620.0	"
0.4	1296.0	"
0.5	1188.0	"
1.0	902.6	"

Table 13: Effect of trace elements on the yield of clavine type of ergot alkaloid.

Isolates	Trace elements	yield in mg/L	Incubation period in days
2C	No trace element	486	10
2C	Only $FeSO_4$	907	"
2C	$FeSO_4 \pm MnSO_4$	1512	"
2C	$FeSO_4 \pm MnSO_4$ $\pm ZnSO_4$	1296	"
2C	$FeSO_4 \pm MnSO_4$ $ZnSO_4 \pm CuSO_4$	1026	"

Table 14 : Effect of incubation period in stationary surface culture for production of clavine type alkaloid

Isolates	Yield in mg/L Incubation period in days		
	30	42	48
2A	1404	1728	1620
2C	1512	1836	1672

Summary

Ergot alkaloids based on chemical structure are mainly divided into two classes : lysergic acid derivatives and clavine type. Both are obtained from ergot sclerotia grown either naturally or by artificial cultivation. It is possible to produce them under submerged culture conditions by optimising physical and nutritional requirements. Any deviation in optimum factors directly affects the yield of alkaloids. Constant temperature of 25°C±1 and *p*H between 5.2 to 5.5 are the ideal factors. Purified mannitol 4 per cent and 5 per cent of sucrose AR grade are the best source of carbon for LAD and clavine types of alkaloids respectively. Fresh inoculum in comparison to older ones proved to be better. Potassium dihydrogen phosphate 0.1 per cent is superior than potassium monohydrogen phosphate for the alkaloids production. Mangnesium sulphate (0.6 per cent) is better for both types of alkaloids. In comparison to calcium nitrate and sodium nitrate as the inorganic nitrogen source, 0.2-0.3 per cent calcium nitrate is better suited for both types of alkaloids under submerged culture conditions. In a comparative study of eight carboxylic acids for the organic source of nitrogen, succinic acid (0.3 per cent) is more suitable for the production of both types of alkaloids. The combination of two trace elements namely, Maganese sulphate and Ferrous sulphate are proved to be better than individual or any other combination for the production of clavine type of alkaloids. Moreover, a combination of all the four trace elements are required for production of lysergic acid derivatives.

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*Ajmalicine (Raubasine) : A Medicinally Important Alkaloid from *Catharanthus roseus (Vinca rosea)*

Jagdev Singh

Regional Research Laboratory
Jammu Tawi

Introduction

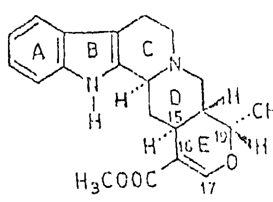
CATHARANTHUS ROSEUS (L.) G.Don is often referred as *Vinca rosea* (Family: Apocyanaceae). In different parts of India it is known as Sadabahar, Sadaphul, Nayantara, Rattanjot, Bilagaanneru, Gul Feringhi, Ainskati and Sudukadu Mallikai[1]. It is pantropical in its occurrence and is widely cultivated as ornamental in the gardens throughout the world. It has been found that the alkaloidal content of *C. roseus* varies considerably in various parts of the plant from different localities and the total alkaloidal content reported [1,2] in the roots ranges from 0.15-1.34% and even upto 1.7948% in some strains. The plant contains more than 100[3,4] alkaloids of the indole group out of which about 25 are dimeric in nature. Two of the dimeric alkaloids vinblastine and vincristine have found extensive application in the treatment of human neoplasms[3]. Among the monomeric alkaloids ajmalicine (raubasine) has been found to have a broad application in the treatment of circulatory diseases, especially in the relief of obstruction of normal cerebral blood flow. In combination with the Rauvolfia alkaloids it has been used to lower high blood pressure. It is reported by Jagdev Singh that 3500 kg[5] of ajmalicine is isolated from natural sources by pharmaceutical industries the world over. Recommended[6] doses for ajmalicine vary widely from 1-20 mg (3 or 4 times daily) by oral or parentral route. The various proprietary products of ajmalicine marketed in European coun-

*Revised and updated

tries are Circolene, Isoarteril, Raubasil, Sarpan (Italy), Hydrosarpan, Raubaserp (Canada), Hydrosarpan Fort (Canada, France), Lamuran (Germany, Italy) and Melanex (S.Africa). Due to the clinical importance of the oncolytic dimeric alkaloids present in the leaves and hypotensive monomeric alkaloids present in the roots, *C. roseus* has attained a high place both in medicine and commerce. The bulk[2,7,8] of the leaves which were earlier exported to USA and Hungary are now being processed[7] by M/s Southern Herbals, Bangalore for the production of vinblastine and vincristine based on the process of NCL, Pune. The firm has for the first time dispatched a first consignment of vincristine (330 g) to Germany fetching about Rs. 3.5 crores. The same firm has also taken up the process know-how from RRL, Jammu for the production of ajmalicine from roots. The roots are also exported[9] to Australia, France, Germany and U.K. and during April-March, 1992 (270, 770 kg) fetched Rs. 9,147, 653 where they are possibly used for the extraction of serpentine and ajmalicine. In order to maintain the supply, the plant is now being cultivated in about 1,500 hectares, mainly in Tamil Nadu and other South Indian States[1].

Chemistry of Ajmalicine

It has been found to occur as a natural product in 20 species of the genus *Rauvolfia*,[1,3] 4 of *Catharanthus*, 2 of *Mitragyne*, in *Pausinystalia yohimbe* and *Stemmandenia obovata*. The yield of ajmalicine reported[1] in *Catharanthus* varies from 0.034- 0.1%. It was first isolated by Siddiqui and Siddiqui[10] from *Rauvolfia serpentina* Ajmalicine belongs to the group of ring E oxygen heterocyclic alkaloids bearing the structure (7). Other alkaloids of E ring oxygen heterocycles[11] include the anhydronium bases, such as serpentine (8) present in *C. roseus* in a yield[1] ranging from 0.02-0.1%. Ajmalicine on dehydrogenation with lead tetra acetate affords the naturally occurring serpentine that in turn can be reconverted by catalystic reduction to the starting substance. The principal difference between the two being in configuration[11] i.e. a trans D/E ring juncture to the ajmalicine group and a *cis* one to the serpentine group. Ajmalicine has also been synthesized [12-18] as a dl mixture and as (-)-ajmalicine [19,20].



I. AJMALICINE
II RING C AROMATIC-SERPENTINE

Production of Ajmalicine

Till recently some Indian firms were marketing only the roots of *C.roseus* or their total alkaloidal concentrates to European countries where these are possibly used for the production of ajmalicine (raubasine). It is commercially useful to export a high value low volume product ajmalicine instead of low value high volume thing

Flow Sheet Showing Isolation of Ajmalicine (Raubasine) and Conversion to Serpentine of Ajmalicine

Powdered *Catharanthus roses* dry roots

Extract with alcohol

Rasidue

Extract with aq.tartaric acid

Aq.acid extract

Extract with ethylene dichloride

Residue (A)

(i) Extract aq. tartaric acid
(ii) Extract $CHCL_3$

Residue

Ajmalicine (0.025%)

Aq. acid extract

(i)Make weakly alkaline
(ii)Extract with ethylene dichloride

Residue (B) (discard)

Aq.alkaline extract

(i)Make strongly alkaline
(ii) Extract with $CHCl_3$

Residue(C) containing mainly serpentine

Aq.alkaline ext. (discard)

Residue (D)

chromatograph over acid alumina using $CHCl_3$:MeOH

Reduce with $NaBH_4$ → Ajmalicine (0.20%)

like roots. Thus Regional Research Laboratory, Jammu took up investigations in this direction and has successfully developed a process on pilot plant scale for the extraction of ajmalicine and serpentine from *C. roseus* and conversion of serpentine to ajmalicine and giving an overall yield[27] of 0.15-0.2% depending upon the percentage of total alkaloids in the roots. The consultancy know-how of the process has been released to six Indian firms (Kothari Chemicals, Madurai, Indo-German Alkaloids, Bombay; Mehta Pharmaceuticals, Amritsar; Cipla Laboratories Bombay; Malabar Phytochemicals, Kerala and Southern Herbals, Bangalore).

The extraction process as reported[27] (Flow Sheet) by us earlier is based on the methodology of selective [28] gradient *p*H extraction technique.

Powdered *C. roseus* roots are extracted in a soxhlet with alcohol. The alcohol from the extract is removed and the residue obtained is extracted with tartaric acid solution. The aqueous acid extract is fractionated into strongly acid, weakly basic and strongly basic fractions. The strongly acid fraction on purification gives ajmalicine in a yield upto 0.025%.

The strongly basic fraction after extraction with $CHCl_3$, removal of the $CHCl_3$ and chromatography of the residue over acid alumina gives crude serpentine in

$CHCl_3$: MeOH eluates. The crude serpentine so obtained is reduced with $NaBH_4$ to give ajmalicine (raubasine) in quantitative yield.

The main equipment required for the process includes hot extractor, liquid extractor, *p*H meter, heating mantle, stirrer, water-bath, distillation assembly and refrigerator.

Purity parameters:

i.	Mol. formula[21]	.	$C_{21}H_{24}N_2O_3$
ii.	Mol. wt.[21]	.	352.42
iii.	m.p.[21]		250-52 (prisms from MeOH).
iv.	$[\alpha]D^{20}$	:	-60° (C = 0 5 $CHCl_3$ -45° (C = 0 5 pyridine) -39° (C = 0 025 MeOH)
v.	pka[22]	:	6 31 (67% DMF in H_2O)
vi.	Rate of methiodide formation[22] $\times 10^{-4} sec^{-1}$ MeOH[21]	.	23.5
vii.	Max	.	227, 292 nm (log Σ 4 61,3.79)
viii.	Cd curves [23]	.	Observed between 250 & 280 nm.
ix.	Infrared spectrum[22,24] cm^{-1}	:	2850-2750 (Bohlman bands), 1700 (C = O), 1620 (C=C).
x.	NMR spectrum[24] ($CDCl_3$)	:	1.12 (d, 3H, J=6.5HZ, CH_3), 3 66(s, 3H,OCH_3), 4.33 (m, 1H, largest J=6.5Hz, $OCHCH_3$), 7.0-7.6 (4 Aromatics), 7.48 (d, 1H, J 1Hz; OCH =), 8.21 (b, 1, NH).
xi.	^{13}C NMR spectrum[25] (ppm upfiled from CS_2)	:	C_2(58.1), C_3(132.5), C_5(139.3), C_6(170.7),C_7(84.6), C_8(65.3), C_9(74.5),C_{10}(71 2), C_{11}(73.27), C_{12}(81.7), C_{13}(56.6),C_{14}(159 7), C_{15}(161.9),C_{16} (85.8),C_{17}(37.9), C_{19}(118.8),C_{20}(151.6),C_{21}(135 7), C_{22}(25.0), C_{23}(141.7).
xii.	Mass spectrum [24,26]	:	m/e 352 (M+), 351 (M-1), 184,169, 156 (b p.)
xiii.	Derivatives[21] a.Ajmalicine HCl	.	$C_{21}H_{25}Cl\ N_2O_3$, leaflets from ethanol, m.p. 290°(dec.) $[\alpha]D^{20}$ -17 (C=0.5 in MeOH)sparingly soluble in water or dil. HCl.
	b.Ajmalicine HBr	.	$C_{21}H_{25}\ Br\ N_2O_3$, diamond shaped platelets from MeOH, m.p 295-96°.

Plant Tissue Culture and Biosynthesis

The alkaloids serpentine and ajmalicine have been produced[29] in cell suspension cultures of *C. roseus* in various laboratories and studied extensively in terms of production and/or biosynthesis. For large scale production Zenk's[30] alkaloid production medium was employed. The alkaloid production varied with the cell line and age of the subculture and ranged from 0.1- 1.5% of cell dry wt. All subculture of cell lines were grown in 7.5 L Microferm bioreactors over a period of 8 weeks. Most cell lines showed a maximum of accumulation in 3rd to 5th week of culture. For processing for alkaloids water is removed by freeze drying and the alkaloids are extracted in the conventional manner. The studies show that the percentage of alkaloids ajmalicine and yohimbin per gram of cell weight increases with time with optimum production at 3-4 weeks.

Formerly pathways of indole alkaloids formation in higher plants were elucidated *in vivo* only by tracer methods. However, the rapid develoment of cell suspension culture techniques in recent years has provided the basis for the reinvestigation of alkaloid biosynthesis[31] at the cell free level. Thus, in combination with radio-immunoassy technique, it was possible to establish cell suspension cultures of *C. roseus* which were capable of synthesising substantial amounts of *corynanthe* alkaloids ajmalicine and serpentine. Using cell strains selected for higher content of ajmalicine as an enzyme source, the precursor role of tryptamine(1) and secologanin(2) in the biosynthesis of ajmalicine and related alkaloids was confirmed *in vitro*. This reaction (Fig.1) is catalysed by enzyme strictosidine synthase, which specifically controls the C-ring closure mechanism. By the action of a second enzyme, a glucosidase, strictosidine(3) is converted via highly reactive intermediates to 4,21-dehydrocorynantheine aldehyde (4,D-ring closure). This aldehyde in turn cyclises (E- ring formation) probably after isomerisation (5) to the next stable intermediate cathenamine (6) 20,21-didehydro ajmalicine.

Finally, the NADPH dependent cathenamine reductase catalyses the synthesis of corynanthe type alkaloid ajmalicine(7) which finally oxidises to serpentine (8).

Zenk[30] *et al.* have indicated the l-tryptophan can be incorporated directly and probably enters the cells more easily than tryptamine.

Lee[32] *et al.* showed that several carotenoid inducers were effective in promoting indole alkaloid formation in *C. roseus* cell cultures with a maximum increase of 20% in ajmalicine and cathranthine. Findings by Kutney [33] *et al.*, have further supported that bioregulators dimethyl piperidium chloride (DPC) and 2- (3,4-dimethyl phenoxy-triethylamine) (DMPTA) caused a significant increase of both alkaloids in *C. roseus* strain.

Kutchan[34] *et al.* have obtained c DNA for strictosidine synthase from *Rauvolfia serpentina* cloned and expressed in *E. coli* and this achievement represents a major break through in plant biotechnology.

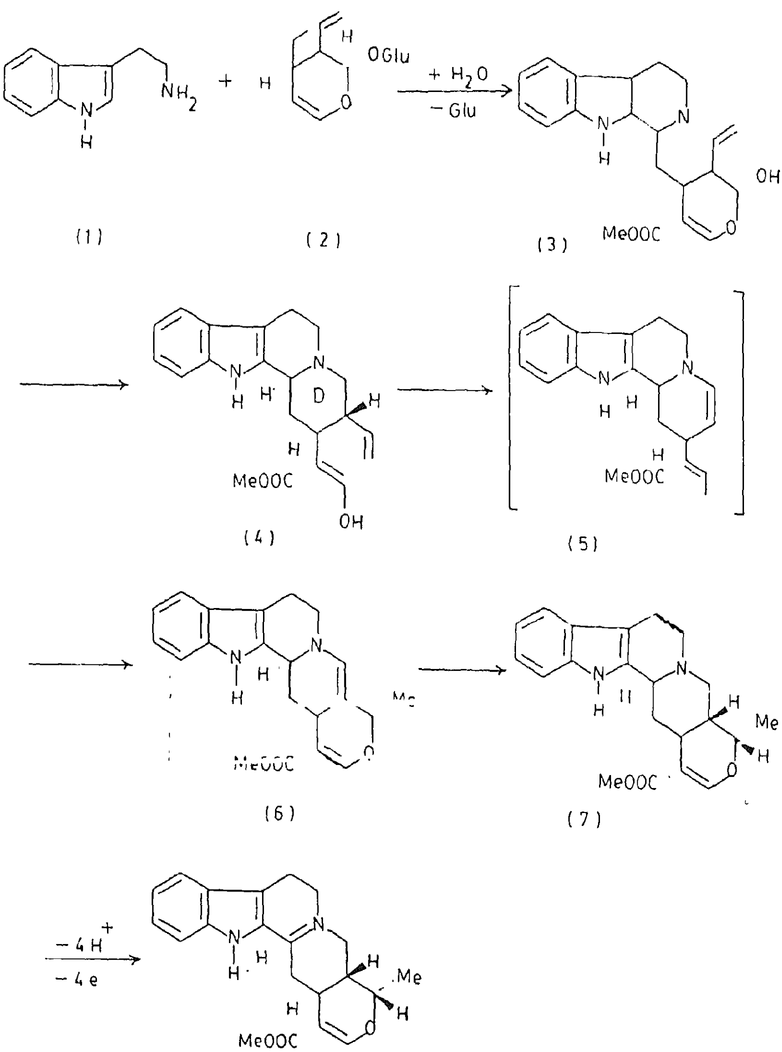


FIG. 1.

Analytical methods

Sarin[35] *et al.* have reported a spectrophotometric method for the estimation of ajmalicine in *C. roseus* roots and stems. The method is based on the quantitative separation of weak bases followed by isolation of ajmalicine by TLC and its determination spectrophotometrically with a recovery of 99± 3%.

Rao[36] *et al.* have further reported a spectrophotometric method for the estimation of serpentine in *C. roseus* roots and stems. The method involves the quantitative separation of serpentine by preparative TLC and its estimation at 310 nm. It also involves conversion of serpentine to ajmalicine and its estimation as ajmalicine at 283 nm.

Zenk[30] and co-workers developed a semiautomatic RIA method for the quantitative determination of serpentine and ajmalicine detecting in the range of 0.1-50 mg/01 ml sample meaning thereby that average alkaloid content of about 1 root cell of *C. roseus* could be quantitatively determind.

Kutney[29] *et al.* used HPLC (High Perfomance Liquid Chromatography) for monitoring subculture development of the target compound in the range of 10-20 mg and has reported it to be the method of choice over radio-active assays. HPLC affords information about the number and concentration of components formed within a given series of fermentation studies.

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Cultivation and Utilization of *Duboisia* spp. in India

Y. N. Shukla

Central Institute of Medicinal and Aromatic Plants,
Lucknow - 226 015

Introduction

Duboisia is endemic to Australia[1]. The genus *Duboisia* (Fam. Solanaceae) is represented by three species, viz., *D. myoporoides* R. Brown, *D. leichhardtii* F.Muell. and *D. hopwoodii* F. Muell. *D. myoporoides* occurs along the eastern coast of Australia and in New Caledonia, *D. leichhardtii* has a restricted occurrence in the highlands of south-eastern Queensland, and *D. hopwoodii* is confined to the interior of the Australian continent[2]. The first two species are rich in hyoscine and hyoscyamine, whereas the third one contains tobacco alkaloids, nicotine and nor-nicotine[1]. Recently, roots of *D. hopwoodii* have been found to contain substantial amounts of hyoscine and hyoscyamine; these are absent or merely detected in leaf[3]. *D. myoporoides* has also been found to contain nicotine and nor-nicotine[2,4]. Thus *D. hopwoodii* and *D. myoporoides* contains alkaloids of tropane as well as pyridine group.

Duboisia sp. have considerable prospects for replacing, or at least supplementing *Atropa*, *Datura* and *Hyoscyamus* as sources of hyoscine and hyoscyamine which are used extensively in medicine throughout the world because of their mydriatic, antispasmodic and anticholinergic properties. The homoeopaths use the tincture and the alkaloid in paralysis and eye infections. So far, the only sources of these alkaloids available in India are species of *Datura*, *Physochlaina praealta* Miers, *Atropa belladona* Linn. and *A. acuminata* Royle. Seeds of *D. innoxia* Mill contain 0.2 to 0.3% of total alkaloids (0.1% hyoscine) and leaves of *D. metel* L.

which contain 0.4 to 0.5% total alkaloids (0.25% hyoscine), are the only source of hyoscine in India. Processing of such raw materials is considered uneconomical because of very low alkaloidal contents. Similarly, leaves of *Physochlaina praealta* (alkaloids 0.6 to 0.7%) and *Datura stramonium* L. (0.3 to 0.8%) are the only source of hyoscyamine available in India. Recently, Egyptian Henbane (*Hyoscyamus muticus*) has been introduced in India which possess 1.3 to 1.5% of total alkaloids. It is a rich source of hyoscyamine and contains traces of hyoscine.

Leaves of *D. myoporoides* contain 2 to 4% of total alkaloids with about 60% hyoscine and 30% hyoscyamine and are the main source of tropane alkaloids in the world. Commercial cultivation of *Duboisia* is done only in Australia, mainly in Queensland area, from where it is exported to Europian countries for further processing[5]. Cultivation of *Duboisia* spp. has also been successfully carried out in Japan[6,7].

First attempt of introducing *D. myoporoides* in India was made several years ago by the Forest Research Institute, Dehra Dun (U.P.) where few trees still exist[8] Central Institute of Medicinal and Aromatic Plants, Lucknow, is trying to establish it as a commercial crop in Northern Plains and Karnataka Its large-scale commercial cultivation would turn India into a major supplier of tropane alkaloids and the country would earn a handsome amount of foreign exchange.

Analysis of leaves harvested after ten months indicate that alkaloid content in *D. myoporoides* grown at Lucknow, varies from 1.90 to 3.56% (approximately 54% hyoscine and 36% hyoscyamine) whereas at Bangalore it varies from 2.75 to 4.90% (56% hyoscyamine and 23% hyoscine)[9]. Such variations in alkaloid spectrum might be due to differences in climatic conditions of north and south. These observations are similar to those obtained in Australia, where similar results have been obtained in north and south Queensland[10].

Botany

D. myoporoides is a tree and may grow as tall as 14 m. As the bark of the trunk is corky and wood extremely light, it is named as corkwood tree. The leaves are broad lanceolate to obovate glabrous. It flowers in spring; flowers are small, white and bell shaped with occasional mauve streakings in the throat of the corolla and are borne in cymose panicles[5]. The fruit is a small black globular berry, about 5 mm in diameter. Flower petals are rounded[11].

Soil and Climate

In nature, the trees grow in cooler and drier climate and require an annual rain fall of 760 mm. They have been found growing on a wide range of soils from the heavy, red clay soils to heavy, dark brown forest loams, to the red and red brown sandy loams. Such soils have a *p*H varying from 4.5 to 5.5 and have low contents of calcium and phosphorus[11].

Good soil drainage is very important. The plant is not a good tolerant of wet conditions. Therefore water-logging should be avoided. As the plants are susceptible to frost in the early stages of growth, a warm site, where good air drainage is assured, should be selected. The site should also be protected from strong winds.

Propagation

The plant is propagated through root cuttings and seeds. The seeds are treated with gibberellic acid having a concentration of 250 ppm, for 24 hrs. and at a temperature of approximately 40°C. Thereafter the seeds are washed thoroughly with water to remove the chemical[9]. Excess of chemical has been found to cause distortion and elongation of the stems at germination. The seeds are planted after 6 weeks following the treatment as the germination percentage is best if left for such period Treated seeds are sown in polythene bags. They germinate in 3 to 4 weeks and field planting takes place in about 10 to 12 weeks if the conditions are favourable for planting. At this stage the seedlings attain a height of 15 to 22 cm and finally they are planted in the field at three spacings (2×1 m, 2×2 m and 3×3 m). Farm yard manure (5 t/ha) and N, P, K at the rate of 50 kg N, 75 kg P_2O_5 and 75 kg K_2O/ha in the form of urea, single superphosphate and muriate of potash are applied before planting in pits.

Light has no effect in seed germination. Seeds stored under dry conditions possess higher germination capacity for about 4 months. For establishment of a new plantation, the soil is ploughed to a depth of 20 to 25 cm, about 2 to 3 months before planting. Autumn is considered to be the best for transplanting. Generally plants are spaced as wide as 4.5 m× 3.6 m. Care should be taken that the seedlings are planted in the field at the same depth as they were in nursery, with a view to protect the seedlings from the crown rot.

Weed Control

Growth of young seedlings is effected by the presence of weeds. Therefore, their control is necessary during the first twelve months. Weeds are generally controlled by discing or chisel-ploughing. Deep ploughing should be avoided. Hilling up around the base of young plants should be avoided as this results in the collar rot disease at ground level[11]. Trifluralin is used as preplant preemergent weed control. If weeds reappear, paraquat is used. Spraying the trunks of young trees should be avoided till the corky brown bark, up to about 30 cm from the ground level, is formed. Weed growth between the rows can be controlled after the first twelve months either by slashing regularly or by inter-row cultivation.

Harvesting

Usually three harvests are obtained in early two years, but the number of harvests depends mainly on season. When conditions are favourable, first harvest is obtained from the new plantations after 7 to 10 months. Succeeding harvests are obtained at

about 7 to 8 months intervals. During harvesting, it is advisable to leave 20 to 30% of leaf on the tree as the regrowth is more rapid if this is done. Yields vary considerably, where 740 to 980 trees are planted per hectare, the average yield of leaf is about 450 kg per hectare. This plant population is within the most favourable yield range and heavier or light planting often results in lower yields.

It was observed that plants grew well both at Lucknow and Bangalore and were ready for harvesting after ten months of growth.

However, the plants are effected by a number of diseases and insects during rainy season at Lucknow. During this period, growth was completely checked and leaves turned yellow. High rainfall, humidity and temperature during rainy season are considered to be the major factors for poor growth, diseases and insect attack at Lucknow. These observations are similar to those reported earlier[12].

The average per plant dry leaf yield (total of three harvests) was 918g at Bangalore as against 318g at Lucknow. The yield obtained at Bangalore was found to be higher than those obtained in Australia (460-600g/plant)[10,11]. The lower leaf yield at Lucknow was attributed to poor plant growth, particularly during rainy season. Different plant spacings did not effect the leaf yield significantly. The alkaloid spectrum in north and south India differs considerably. In the north the alkaloid contents were lower with more hyoscine than hyoscyamine whereas in the south it was higher with more hyoscyamine than hyoscine. As the climatic conditions at Bangalore were found very much favourable, a plantation of more than 200 trees has been established at Kodimanchanahalli in Bangalore where these are growing very well.

Hybrids of Duboisia

Australian workers have successfully grown the hybrid plants of *D. myoporoides* and *D. leichhardtii* in hydroponic culture and studied the nutritional effects of nitrogen, phosphorus and potassium. Double the amount of nitrogen in standard nutrient gave a significant increase in the size of the plants. The total alkaloid content was about 27% lower than that found in control plants. Any further increase in nitrogen resulted in reduction of total alkaloid by 62%. Increased potassium showed a significant increase in the percentage of hyoscine[13]. Effect of growth regulators on alkaloid contents have also been studied. Plants treated with 6-dimethylaminopurine, 6-benzylaminopurine (BA) and a commercial seaweed extract, Maxicrop, all exhibited an increase in the percentage of total alkaloids. Hyoscyamine was increased highly significantly when plants were treated with BA at 5 ppm and 6-dimethylaminopurine at 1 ppm. A significant increase in the percentage of hyoscine was observed in plants treated with a strong solution of Maxicrop. A weaker solution of Maxicrop gave a highly significant increase in the percentage of hyoscyamine. In commercial plantation spraying with Maxicrop showed an 18% increase in leaf yield and a 16% increase in hyoscine content as

compared to that of the control[14]. There was no significant increase in total alkaloid content.

Chemistry

Tropane and nicotine alkaloids are usually found in *D. myoporoides*. They are present in various parts in different percentages[15]. The tropane alkaloids occur as esters with acetic, butyric, valeric, tiglic, α-methylbutyric acids[16] and C_9 acids, viz. tropic, cinnamic, phenyl lactic and nonanoic acids. Apoatropine, atropine, apohyoscine, anabasine, butropine, hyoscine, hyoscyamine, isopelletierine, isoporoidene, nicotine, nor-nicotine, nor-atropine, nor-hyoscine, nor-hyoscyamine, pelletierine, poroidene, tetramethylputrescine, tigloidine, tropine, tropyl acetate, valtropine and valeroidine have been reported earlier from *D. myoporoides*. The plant grown in India showed the presence of apoatropine, butropine, hyoscyamine, hyoscine, valtropine,6β-hydroxyhyoscyamine and a new base tropine nonanoate[17]. This new base has been shown to possess antimuscarinic activity which is of low order as compared to standard drug atropine sulfate. This activity was determined by its inhibition of binding of muscarinic antagonist [^{3}H] N-methylscopolamine to bovine brain cerebral cortex.

Among the non-alkaloid constituents it afforded the triterpenoids, ursolic acid and betulonic acid and the lipids, dotriacontanol, tetratriacontanoic acid, 8-hydroxydo-triacontan-30-one[18], 6-methyldotriacontane, hentriacontanyl tetratriacontanoate, 4-pentatriacontanone, and 3-hydroxydotriacontan-28-one[19].

The bases are mainly formed in the roots and are stored in the leaves. GLC has been routinely used for the analysis of hyoscine and hyoscyamine[20]. Nicotine was found to be absent in Indian *D. myoporoides*.

Utilization

Tropane alkaloids are reported to posses antispasmodic[21], mydriatic and anticholinergic properties. Atropine, hyoscyamine and hyoscine are strongly mydriatic When instilled into the eyes of cat, they cause dilation of the pupil in very dilute solution. The drugs which block the action of acetyl choline at parasympathetic endings in general are likely to be mytriatic. They may also paralyse the ciliary muscles so that the eye becomes set for far vision (cycloplegia) Atropine is the classical example of drug which produce both these effects[22] Because hyoscyamine has undesirable actions on central nervous system, hyoscine as hyoscine-N-butyl-bromide is the preferred parasympatholytic agent. Atropine, obtained by the racemisation of hyoscyamine is used as its neutral sulfate in 1% solution When this solution is instilled into the human eyes, mydriasis begins in about half an hour and is complete in an hour; the effect is remarkably prolonged and may not disappear completely for 7-10 days. Hyoscine is more powerful than atropine. It is usually administered as its hydrobromide and a 0.2% solution of this

salt can be used instead of 1% atropine sulfate. Hyoscine hydrobromide also controls motion sickness. Hyoscine-N-butylbromide (Buscopan) has a great demand now a days. It is also claimed to have antispasmodic property.

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Production of Colchicine from *Gloriosa superba* seeds

B.K.Gupta

Regional Research Laboratory
Canal Road,
Jammu - 180 001.

Introduction

Colchicine, the principal alkaloid of *Colchicum autumnale* L. (N.O.Liliaceae) is a biologically active molecule, possessing anti-inflammatory, antimiotic and tumour-inhibiting activities[1]. Several reviews on chemistry and pharmacological properties are available[2-7]. The term Colchicine is derived from the area known as 'Colchis' near the Black Sea. The use of Colchicum in gout[8] was recorded in 560 A.D. and it was so employed until 13th century. In 1873 the re-employment of colchicum in medicine under the preparation "Medicinals" became very popular for the treatment of gout. Early idea of action of Colchicum in gout treatment were pronounced by Thomson and others. In the 19th century it was realised that gout and uric acid metabolism were in some way linked and colchicum might act on this. Gout is caused by deposition of microcrystals of uric acid in joints and may be due to a defective regulatory mechanism for endogenous purine synthesis, but conflicting results for the action of colchicine on synthesis and excretion of urates have been recorded. Colchicine interrupts the cycle of new crystal deposition which seems to be essential for the continuance of acute gout. Distressing side effects have been recorded occasionally but colchicine remains the drug of choice for acute gout. Modification of the side chains of the rings does not abolish anti-gout activity as long as the configuration of C- ring conforms to that of colchicine.

Colchicine may offer a lease of hope to the patients with liver cirrhosis. Experiments carried by Kershenobich *et al.*,[9] showed that oral colchicine, which inhibits collagen synthesis and secretion and enhances collagenase activity, decreased fibrosis and liver malfunction in rats with carbon tetrachloride induced cirrhosis. The results of the study by Kershenobich *et al.*, raised hope for the treatment of cirrhosis at hand. They are not conclusive because of serious flaws[10] in survival analysis and screening process Colchicine has also been advocated in many other diseases like cholera, typhus, Bright's disease, colic and skin complaints

Colchicine acts on mitotically active cell producing metaphasic arrest, the 'c-mitotic' action often results in doubling of chromosome number and giving rise to polyploids[11,12]. This was discovered first by Pernice in 1899 who did not realize its significance. This action was rediscovered in 1930's and was followed by considerable work on the action of colchicine on plant and animal cell and utility of this phenomenon in biology and medicine.

Plant source

Since 1930 the demand of Colchicine has increased which stimulated great interest in plant sources. In this direction much work on resources and extraction of colchicine have been reviewed by Santary *et al*[13-20]. Different genera studied include *Androcymbium* (Africa), *Colchicum* (Europe, Russia, and India), *Gloriosa* (India, Ceylon and Africa), *Iphigenia* (India and Africa), and *Merendera* (Europe and India). It is quite interesting to know that *Colchicum cornigerum*[21], a species native to Egypt, has provided the only example which contains colchicine related alkaloid but no colchicine as such. Colchicine is manufactured from *Colchicum autumnale*, a plant indigenous to and widely met within the European continent. A worldwide search for alternative plant sources have been under way. Amongst the Indian plants the corms of *Colchicum luteum* and seeds of *Iphigenia stellata*[22-25] reported to be rich in colchicine content, are not available in sufficient quantities to warrant any commercial utilization. *Gloriosa superba* L. is another plant containing Colchicine. Many workers[26-34] have isolated colchicine, colchicine related alkaloids and non-alkaloidal contents from the various parts of this plant. In continuation to our work on indigenous sources of colchicine, extensive analytical studies on the various parts of this plant revealed that the percentage of colchicine in seeds[35] is ten times more than that in the tubers. This prompted us to explore the large scale isolation of colchicine in India.

Description of Plant

Gloriosa superba Linn. (N.O.Liliaceae)[36] is a perennial herbaceous climber occurring among scrub forests throughout India upto an altitude of 1800m. It is native of tropical Asia and Africa and is commonly known as Glory Lily or Malabar glory

Lily. The plant flowers during July and August while the capsules (5-6 cm long) containing ripe seeds can be harvested by the end of September. Propagation is effected by tuber cuttings which are planted in the month of May and sprout in July. The fruits with ripe seeds are ready for harvest in the first week of October. The high colchicine content of the seeds accompanied by prospects of their good availability from both wild and cultivated sources, make the material a potential commercial source of colchicine in India.

Exploitation of seeds as source of colchicine has following advantages over the Colchicine extraction from tubers:

1. Perennial nature of the plant ensures sustained supply of the raw material over a number of years.
2. Colchicine content of the seed is ten times more than that in the tubers.
3. The process developed from the seeds is very facile as eliminates the use of column chromatography completely.
4. The recovery of the colchicine content from the seeds is higher than that from the tubers.

Extraction method

Toxic principle of *Colchicum autumnale* was first isolated by Pelletier and Caventou in the year 1819 which believed it to be veratrine. The extraction method of colchicine alkaloids developed by Santavy *et al.*,[37] has given poor yield of colchicine when tried on tubers of *Gloriosa superba*.

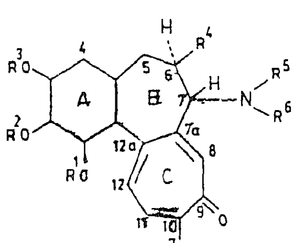
A process for the extraction of Colchicine ex *G. superba* tubers was developed based on high solubility of Colchicine in water[37a]. The total alkaloids, thus obtained by water extraction, were further purified through partition with Chlorinated solvent followed by column chromatography over neutral alumina to yield pure colchicine and related alkaloids. Since the seeds of *G. superba* are rich source of Colchicine, attempts were made to develop an economically viable method for the production of colchicine by using various solvents and other parameters such as temperature, time etc. Aqueous ethanol was found to be the solvent of choice for the extraction of the alkaloid from the de-fatted seeds. The crude colchicine thus obtained was purified by using mixtures of solvents instead of column chromatography. The process was found to be successful on large scale trials. The detailed process is given below:

The de-fatted moderately powdered seeds are extracted with continuous stirring using aqueous ethanol as solvent till last drops of the extract do not give positive test for the alkaloids. The total extract is combined and filtered to remove the sediments. The clear filtrate thus obtained, is concentrated under reduced pressure to 1/6th of the total volume. The concentrated golden yellow solution, thus obtained, is further extracted with chloroform with the help of liquid-liquid extrac-

tor. The total chloroform extract is dried over anhydrous calcium chloride, filtered and distilled under reduced pressure to yield yellowish green semi-solid mass (1.5%) as neutral-phenolics. The resinous mass from the neutral-phenolics is removed by treatment with a mixture of chloroform and petrol and colchicine crystallised from ethyl acetate to yield 0.70% creamish yellow crystalline material which is highly soluble in methanol, ethanol and water, m.p 150- 3°.

Chemistry and Structural Relationship of Colchicine with other Related alkaloids

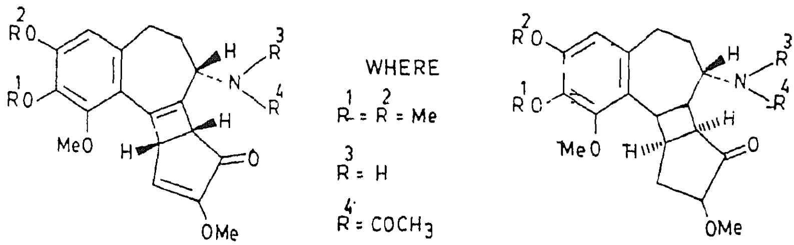
Chemically, colchine is named as methyl ether of colchicine. Furthermore colchicine, N-formyl-N-desacetyl colchicine and 3-demethyl colchicine designated as substance A,B and C respectively have been reported to occur in various plants of liliaceae family (Fig 1). Chemical studies on these alkaloids indicated the presence of a methoxyl group in colchicine possessing only asymmetric carbon at position C-7 This seems exceptionally simple when compared with complex alkaloids such as morphine and strychnine yet structural studies spanned more than 100 years The total synthesis of colchicine was not achieved until 1959. The structure of colchicine does possess some unusual feature of which seven membered ring B and more particularly the troponoid ring C, deserve special mention. The structure of colchicine has attracted widespread attention as a challenging synthetic problem [Schreiber *et al.*[38], Tamelen *et al.*[39], Nakamura *et al.*[40], Scott *et al.*[41], Woodward[42], Kotani *et al.*[43], Evans *et al.*[56], Boger *et al.*[57]Ik and Brossi *et al*[58],]. When Colchicine is irradiated by light, photoisomerization occurs and α- β and γ-lumi-derivatives are formed. Santavy isolated compounds designated as I- and J-substances from the natural sources along with the colchicine. The physical properties of compounds I- and J-correspond to β- and γ- lumicolchicine respec-



COMPOUND	R^1	R^2	R^3	R^4	R^5	R^6	R^7
I COLCHICINE (A)	CH_3	CH_3	CH_3	H	CH_3CO	H	CH_3
II N-FORMYL-N-DEACETYL COLCHICINE (B)	CH_3	CH_3	CH_3	H	CHO	H	CH_3
III 3-DEMETHYLCOLCHICINE (C)	CH_3	CH_3	H	H	CH_3CO	H	CH_3

FIG. 1

tively. NMR [44] studies of colchicine and its photoisomers, β- and α-lumicolchicines have been obtained using a combination of 1D- and 2D-nmr spectroscopic techniques. The study revealed that γ-lumicolchicine is a steroisomer of β-lumicolchicine shown in Figure 2.



IV β-LUMICOLCHICINE V γ-LUMICOLCHICINE

FIG. 2

Biosynthesis of colchicine

Colchicine (I) is an alkaloid with a unique tropolone ring and on the face of it appears to be quite unrelated to any other alkaloid. A pioneer work on the biosynthesis of this alkaloid was carried out by Battersby *et al.*, and Leete *et al.*, on the different species of *Colchicum* and accordingly acetate was the precursor of the N-acetylgroup[45,46]. Phenylalanine by way of Cinnamic acid serves in *Colchicum* species as the source of ring A plus carbons 5,6 and 7. The seven Carbons of the tropolone ring arise from tyrosine which loses C-1 and C-2 from the side chain labels from [4'-^{14}C] and [3-^{14}C] labelled amino acid appear at C-9 and C-12 of colchicine respectively[47]. It follows that the tropolone ring arises by expansion of the tyrosine benzene ring with inclusion of the benzylic carbon but through what series of intermediates.

The answer came from the elucidation of the structure of androcymbine an alkaloid isolated from a relative of *Colchicum*. The dienone structure (VIII) was assigned to androcymbine. Androcymbine could then be thought of as arising from the phenethylisoquinoline skeleton [VI] which was quite unknown at the time[48]. A similar biogenesis for colchicine followed. Plausibly the transformation of the androcymbine skeleton [VIII] to that of colchicine could occur by hydroxylation to give [IX]. Homoallylic ring expansion would lead to colchicine (shown in Figure 3).

A crucial test for the hypothesis lay in the examination of compounds of type [VI] & [VIII] as Colchicine precursors. In the event [49-51], O-methyl androcymbine

BIOSYNTHESIS OF COLCHICINE

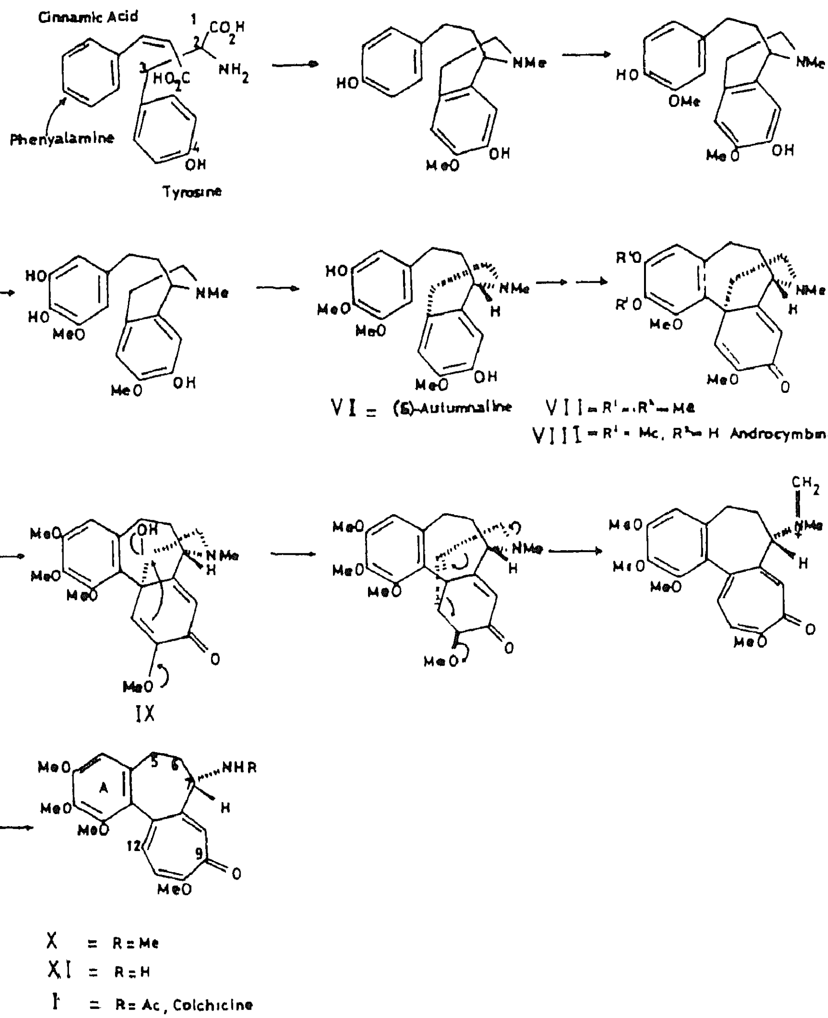


Fig. 3

[VII] was a spectacularly good precursor for colchicine. The phenethylisoquinoline [VI] called autumnaline, was also clearly implicated in biosynthesis, only the (S)-isomer of autumnaline with the same absolute configuration as colchicine is involved, and oxidative coupling of this base occurs in a *para-para* sense rather than the alternative *ortho-para*. Results of other experiments together with those discussed here, led to the Pathway illustrated in Figure 3. Examination of the final

BIOSYNTHESIS OF PHENTHYLISOQUINOLINE

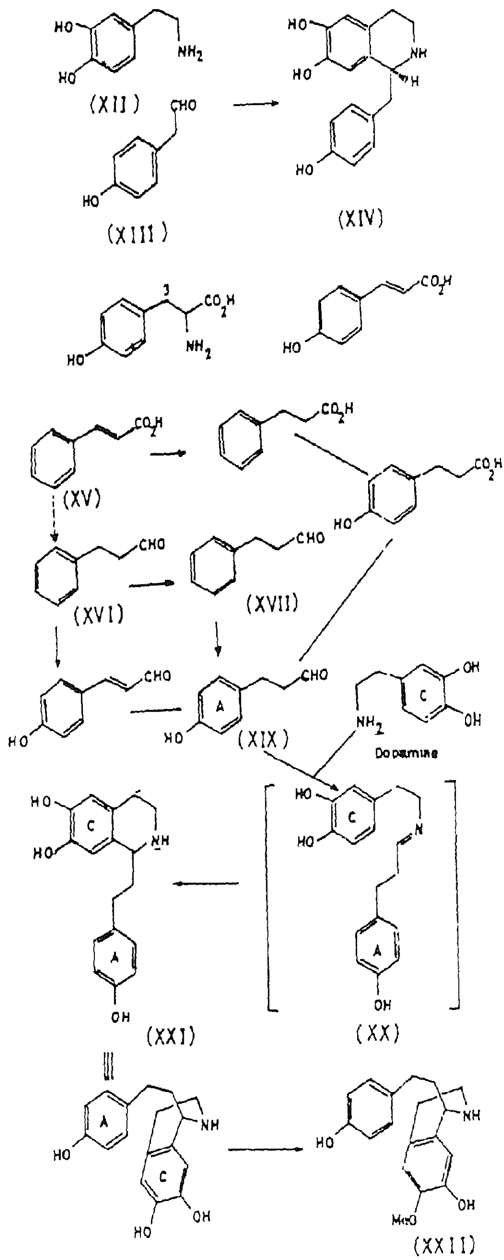


Figure.4

stages of Colchicine biosynthesis showed that demecolcine [X] and deacetyl colchicine [XI] were involved.

It is with exceptions, now a common observation[52,53] for the biosynthesis of very many different alkaloids that a key step involves condensation of an aldehyde with an amine, e.g; biosynthesis of benzyl isoquinoline alkaloids involve the enzyme-catalysed condensation of dopamine [XII] with 4-hydroxy-phenylacetaldehyde [XIII] yielding (S)-nor Coclaurine [XIV] which is the precursor then for the whole family of benzylisoquinoline alkaloids[52]. Keeping in view this observation Herbert *et al.*[54], recently conducted feeding experiments with mixtures of precursors where one compound bore ^{14}C-label and the other ^{3}H- label. The combined results indicate the course of biosynthesis for colchicine [I], demecolcine [X] in *Colchicum* sp. (Liliaceae) illustrated in Figure 4.

In analogy to (S)-nor Coclaurine, a precursor for benzylisoquinoline alkaloid, from cinnamic acid [XV] via cinnamaldehyde [XVI] arises dihydrocinnamic aldehyde [XVII] which generates an important intermediate as a precursor identified as 3-(4- hydroxyphenyl) propanal [XIX]. This precursor allows to build upon the pathway for the biosynthesis of colchicine illustrated in Figure 4. The condensation of the aldehyde [XIX] with dopamine [XII] affords unstable Compound [XXI] Via [XX] which was identified as trihydroxy isoquinoline. The cinnamyl double-bond is reduced before the formation of phenethylisoquinoline skeleton [XXII] similar to structure [VI] shown in Figure 3 which was not known earlier. The results reported indicate that n-methylation need not occur straight after the formation of [XXII] i.e. may occur after aromatic hydroxylation and O-methylation. Thus it is established that phenethyl isoquinoline is an intact precursor for demecolcine and colchicine.

Herbert *et al.*[54]., observed that *Sceletium* alkaloids belonging to the Aizoaceae family are formed in the early stages in a similar pathway to the *Colchicum* alkaloids, i.e. 3-(4-hydroxyphenyl) propanal [XIX] is involved as a precursor as it likely combines with dopamine to form a Schiff's base which acts as an intermediate (XX) in *Colchicum* biosynthesis. It seems as if there is lack of aryl hydroxy group in *Sceletium* biosynthesis which prevents isoquinoline ring formation. This indicates that colchicine has no structural relation with other alkaloids[56].

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Holarrhena *antidysenterica* - A Review

V. Gopal and Mrs. M.G. Chauhan

Department of Pharmacognosy,
L.M.College of Pharmacy,
Ahmedabad.

Introduction

Holarrhena R. Br (Apocynaceae) is a genus of trees or shrubs distributed throughout the tropical and subtropical regions of the world. About eight species of this genus are known but only one "Holarrhena *antidysenterica* (Linn) Wall, synonym Holarrhena *pubescens* (Buch-Ham) Wall grows in India (Trease and Evans 1989). In Sanskrit it is commonly known as Kutaja. The different parts of the plant were used since antiquity in the indigenous system of medicine but the stem bark and the seeds were more extensively employed as antidiarrhoeal and anthelmintic drugs. Seeds are sold in the market under the common name of "Indrayava" while the bark under the names of "Kurchi", "Conessi", "Tellicherry" or "Koora". Its name "Koora" is confusing because some of its commercial adulterants like *Wrightia tinctoria* and *Wrightia tomentosa* are also labelled as "Koora" (Kaul and Atal 1983).

Distribution and Habitat

The plant is found throughout the drier or deciduous forest areas of India at low elevations and ascending to 3500 feet elevation in the tropical Himalayan tract from the Chenab eastwards. It often grows gregariously and is common in the Sal forest, the Aravalli hills south of the Dewair pass, Bihar, Central provinces as well as in South Konkan and Kerala. It can be successfully grown in the most reclaimed waste land with moderate rainfall.

Habit and General Features

Holarrhena *antidysenterica* Wall. is a small to medium size deciduous tree, attaining a height of forty feet or more though often not bigger than a tall shrub, bearing fairly large, opposite, short petioled membranous ovate oblong, prominently veined leaves; cymose clusters of large white somewhat fragrant flowers, and pairs of narrow slender, foot long pendulous follicles, (Fig 1). The shoots when young are often tomentose. The plant produces root suckers in abundance. It flowers at different times at different parts, usually from February to May, or occasionally in June to July, and September to November. The wood is white, soft, even grained and marked with faint annual rings, many very fine medullary rays, and numerous small pores in radial lines.

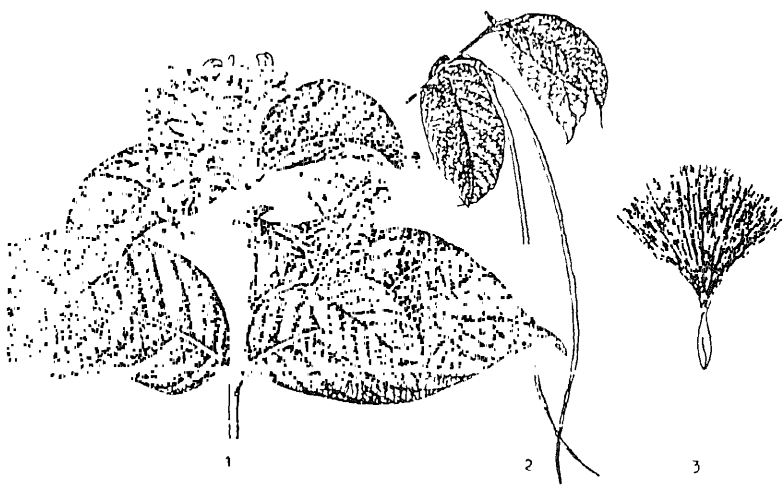


Fig - 1. Holarrhena antidysenterica, Wall.
1. A twig with flowers 2. Fruit 3. Seed

Medico Ethnobotany

The medicinal properties of Kurchi were known as early as 1000 B.C. Ayurveda categorised these properties under the following headings;

Rasa	: Katu, Kashaaya, Tikta
Guna	: Laghu, Rooksha
Veerya	: Sheeta and
Vipaaka	: Katu

On the basis of certain dominant and recessive characters of the plant, Charaka has described two varieties of Kurchi. A plant with smooth leaves, white flowers and bitter tasting seeds was named as masculine while a plant with reddish flowers, smaller fruit and fruit stalk and non-bitter seeds was named as feminine variety. In

vernacular the seeds of masculine and feminine varieties were named as "Kaduva Indrayava" (bitter kurchi seed) and "Mitha Indrayava" (sweet kurchi seed) respectively and it was suggested that the plant with bitter seed only possess medicinal properties. Masculine and feminine varieties are now recognised as Holarrhena *antidysenterica* and *Wrightia tinctoria* respectively.

All systems of medicine recommend the use of the bark as a principal remedy in cases of various types of dysentery. Kirtikar and Basu (1933) mentioned the use of all the parts of the plant except flower in cases of snake bite and scorpion sting. Evidences of chronic cases of dysentery which could not be cured by emetine but cured by the preparations of this bark have been reported in European medical treatment (Nadkarni 1955) and substitution of the Kurchi bark in place of emetine has also been suggested (Nandi and Majumdar 1979).

Jain and Tarafder (1970) have mentioned the use of this plant by the various Indian tribals in cases of number of ailments like anaemia, epilepsy, obstetric conditions, spermatorrhoea, haematuria, constipation, stomach ache, Cholera and in dog bites. Sharma *et al.*, (1979) recommends the seeds in cases of Jaundice. Gopal and Chauhan (1993) have documented the use of Kurchi seed in the treatment of Diabetes mellitus.

In Ayurveda, the plant has been extensively used in the treatment of various bleeding disorders like diarrhoea, dysentery, piles, abortions, invisible hemorrhoids etc. Sushruta has advocated the use of the flowers in prameha (diabetes). Kaul and Atal (1983) have summarized the uses of various parts of the plant in the different systems of medicine as in Table 1.

Besides these systems its use in homeopathic system of medicine has also been mentioned by Nandi and Majumdar (1979). Singh and Chaturvedi (1982) have mentioned its use as a single drug therapy or in combination with other drugs in almost all types of dosage forms, and have cited out about 20 popular formulations containing the bark or seed of Kurchi as one of the major ingredient.

Non Medicinal Applications

This species is important in reclothing waste lands. It acts as a nurse to more valuable species like Sal seedlings in forests. It is also cultivated as an ornamental plant for its beautiful flowers. Chopra (1956) has mentioned the commercial use of the leaves for bidi making and the Wealth of India (1959) mentioned the use of wood for making small articles such as combs, boxes, cups, ploughs, mathematical instruments and furnitures.

Pharmacognostical Studies

Prasad and Kaul (1956) have described in detail the pharmacognosy of Kurchi bark and its adulterant *Wrightia tomentosa* while Atal and Sethi (1962) have described the pharmacognosy of its another adulterant *Wrightia tinctoria*. Characters which differentiate these adulterants from authentic Kurchi bark are summarised in Table 2.

Table 1

Sr. No.	Part used	System of medicine	Uses
1.	Stem bark	Ayurveda	Anthelmintic, stomachic astringent, in cases of diarrhoea, fever, piles, leprosy, thirst, skin diseases, diseases of spleen, dropsy, and biliousness.
		Yunani	Used against headache, strengthens the gums, reduces inflammation and excessive menstrual flow.
		Portuguese	Used as a plaster in rheumatism, as a hot decoction in toothache and bowel infections.
		British Materia Medica	Antidysenteric.
	Root bark	Portuguese	As an antipyretic in cases of long standing fever.
2.	Seed	Ayurveda	Cooling, appetizer, carminative astringent, anthelmintic; in cases of leprosy, burning sensation, dysentery, skin diseases, biliousness; bleeding piles, fatigue and hallucinations.
		Yunani	Carminative, astringent lithon- triptic, tonic and aphrodisiac, used against chronic chest infections, pessaries made with honey and saffron are supposed to favour conception.
3.	Leaves	Yunani	Astringent, galactagogue, tonic, aphrodisiac, mitigates pain in muscles, cools the brain, useful in cases of chronic bronchitis, lumbago, urinary discharges, boils, ulcers, wounds, burns, regulating menstruation and for fumigating the mother and child after delivery.
4.	Flowers	Ayurveda	Appetizer, anthelmentic, anti-hepatotoxic, antidiarrhoeal, and in diseases of blood and leucoderma.

Table 2: Distinguishing characters of Kurchi bark and its adulterants

Characters	Holarrhena *antidysenterica*	*Wrightia tomentosa*	*Wrightia tinctoria*
Macroscopy			
Shape	Recurved	Recurved	Channelled, quilled
Outer surface			
Colour	Buff to reddish brown	Yellow to greenish brown	Light grey
Lenticels	Prominent circular or transversely elongated	Absent	Smaller, circular, whitish
Inner surface	Brown, rough often with pieces of wood attached	Brownish white, and strongly fibrous	Pale brown, smooth
Fracture	Short and granular	Tough and fibrous	Tough and brittle
Taste	Extremely bitter and acrid	Not characteristic	Bland
Microscopy			
Stone cells			
a. Distribution	Throughout the section, arranged in concentric tangential bands in phloem region	Throughout the section	In young, bark-absent, in old present only in cortical region
b. Size (μ)	75-150μ by 30- 60μ	Small:μ 30-80 by 30-70 Big: 45-120μ by 30-60μ	13μ- 51μ by 24μ-41μ
c. Wall	Pitted	Pitted	Striated and highly thickened
d. Calcium oxalate prisms	Present	Absent	Rarely present
Pericyclic fibres	present in young bark	Absent	Absent
Phloem fibres	Absent	Present	Present

Contd.

Characters	Holarrhena *antidysenterica*	*Wrightia tomentosa*	*Wrightia tinctoria*
Phloem Parenchyma	Polyhedral to more or less isodiametric	Rectangular	Irregular
Medullary rays	Bi to triseriate,,very few uniseriate some of the cells sclerosed	Uniseriate, very few bi to triseriate	Uniseriate, few biseriate
Cell contents			
a. Calcium oxalate	Prisms	Prisms	Large prisms of characteristic shapes
b. Starch	Present	Absent	Absent
c. Latex	Cream coloured somewhat transparent, in laticiferous cells	Dark brown, in laticiferous vessels	Dark brown granular, in septate, laticiferous vessels

Khan (1987) studied the comparative morphological and microscopical characters of the seeds of *H. antidysenterica* and one of its adulterant *W. tinctoria* available in the market under the name of "Sweet indrajava". The comparative account of this is mentioned in Table 3.

Alkaloids of Kurchi

Alkaloids have been reported in the bark, leaf and seed parts of the plant. Bark is rich in alkaloids which are located in the phloem tissue and not in the periderm (Datta and Bal 1945). The alkaloidal content of the bark varies with the age and girth of the plant. Eight years old stem bark contains maximum amount of alkaloids (3-4%) and hence are used in the commercial scale production of *Holarrhena* alkaloid hydrochlorides and *Holarrhena* Bismuth iodide (Prasad and Kaul 1957). The Indian Pharmacopoeia (1966) mentioned 2% minimum limit for the total alkaloids in Kurchi bark and 1% w/v in liquid extract of Kurchi. Bhutani *et al.*, (1984) have reported the alkaloidal content of the bark (0.6-3.90%), leaf (0.60-1.4%) and seed (0.30-0.91%) of wildly growing trees from different regions of India, the highest (4.27%) being in the stem bark collected from Gujarat state. The alkaloidal contents of the commercial samples of bark (1-2.3%) and seed (0-0.7%) from the different regions of India were also reported by them. The adulterants of Kurchi bark, *W tomentosa* contain 1.55% (Jayaswal 1977) and 0.4% (Bhutani *et al.*, 1984) of alkaloids while that of *W. tinctoria* contain 0.23% (Bhutani *et al.*, 1984) of alkaloids.

Table 3: Comparative account of some exo- and endomorphic charactersof seeds of Holarrhena *antidysenterica* and *Wrightia tinctoria*.

Characters	Holarrhena *antidysenterica*	*Wrightia tinctoria*
Seed		
Shape	Nearly plano-convex, compressed and oblong	Nearly ellipsoidal, chalazal side is more elongated than micropylar side.
Size	14 to 16 mm long and 3 to 3.5 mm broad	18 to 21 mm long and 2 to 2.5 mm broad.
Hairs	Light brown silky about 45 mm long, present at the micropylar end only	Dull white silky about 54-65 mm long present at the chalazal end only.
Weight of 100 seeds	2.78 g	2.89 g
Seed coat	3 to 5 layers thick except at the hilar region and at the micropylar end where number is more. Epidermal cells thicker and have dense tanniferous deposits	About 7 layers thick and the epidermis is slightly thicker and with less tanniferous deposits
Endosperm	Three layers on lateral sides and more on micro-pylar and chalazal ends	Three to four cell layers on lateral sides and more on the micropylar and chalazal end.
Folding pattern of cotyledons	The cotyledons are large and remain folded in-wardly in a characteristic manner in the seed	The cotyledons are long and leafy and remain folded in the seed in a characteristic convolute folding.
Spermodermal pattern	Tuberculate	Reticulate with interwoven rugae.

Dutta *et al.*, (1950) studied the seasonal variation of the alkaloidal content in the plant. The highest content of alkaloid has been found to be soon after the rains, and in the months of November (stem bark : 3.89% ; root bark : 3.76%) and December (stem bark : 3.78% ; root bark : 3.8%) while the leaf contain highest amount in June (1.56%). The alkaloidal contents of stem (1%) remain constant throughout the year.

Considerable work has been carried out on the chemistry and biological activity of Kurchi which has been reviewed by several authors like ; Roy and Mukherji (1958), Bhandari and Mukherji (1959), Gunnar *et al.*, (1968) and Chatur-

vedi *et al.*, (1980), (1981). A bibliography on kurchi has also been published Anonymous (1982).

Alkaloids isolated from kurchi are listed in Table 4 and the structures of some of them are given in Figure - 2.

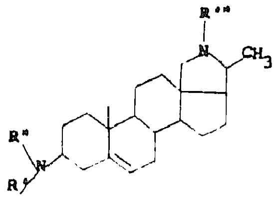
Table 4. List of Alkaloidal Bases Isolated from *Holarrhena* antidyrenterica

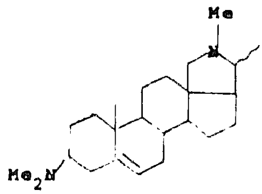
Sr. No.	Name of the alkaloid	Parts of the Plant	Author	Year
1.	Conessine	Bark	Haines	1858
2	Kurchicine	Bark	Ghosh, S., *et al.*	1928
3.	Kurchine	Bark Ghosh, S., *et al.*	1928	
4.	Norconessine	Seeds	Robert, D.H 1932	
5	Conessimine	Bark	Siddiqui *et al.*	1932
6	Holarrhine	Bark	Siddiqui *et al.*	1932
7	Holarrhimine	Bark	Siddiqui *et al.*	1932
8.	Conessidine	"	Bertho *et al.*	1933
9.	Conkurchin	"	Bertho *et al.*	1933
10.	Curchinine	"	Bertho *et al.*	1933
11.	Conimine	Bark and seed	Siddiqui *et al.*	1934
12.	Isoconessimine	Bark and seed	Siddiqui *et al.*	1934
13.	Lettocine	Bark	Peacock *et al.*	1935
14.	Conamine	Bark	Siddiqui *et al.*	1936
15.	Conarrhimine	Bark	Siddiqui *et al.*	1936
16	Holarrhesimine	"	Tschesche *et al.*	1954
17.	Trimethyl Conkurchine	"	Tschesche & Roy	1956
18.	Holarrhidine	Bark	Labler,,I., *et al.*	1957
19.	Kurchamine	"	Tschesche *et al.*	1958
20.	Kurchessine	"	"	1958
21	Tetra Methyl Holarrhimine	"	"	1958

Contd.

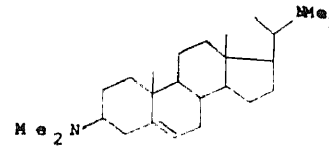
Sr. No.	Name of the alkaloid	Parts of the Plant	Author	Year
22.	(3)-N-Methyl Holarrhimine-2HCl	"	"	1958
23.	(20)N-Methyl Holarrhlmine	Leaflets	"	1958
24.	Kurchimine Kurcholessine	Bark	Tschesche & Peter	1962
25.	Dihydroconcurressine	"	Labler & Sorm	1963
26.	Concuressine	"	"	1963
27.	Epihetroconessine	"	"	1963
28.	Dihydroisoconessimine	"	Cerny *et al.*	1964
29.	3a-aminoconan-5-ene	"	"	1964
30.	7a-hydroxyconessine	"	Tschesche & Ockenfels	1964
31.	Holonamine	"	"	1964
32.	Kurchiphyllamine	Leaflets	Janot *et al.*	1966
33.	Kurchiphylline	Leaves	Janot *et al.*	1966
34.	Kurchiline	"	-	1966
35.	Kurchaline	"	-	1966
36.	Holadysamine	"	-	1966
37.	Holantosines A,B	"	-	1966
38.	Holantosine C, D	"	-	1970
39.	Holarosine-A	"	Qui *et al.*	1971
40.	Holantosines E, F	"	Goutarel Robert	1972
41.	Holarosine-B	"	"	1972
42.	Holacetine	Root Bark	Rej *et al.*	1976
43.	Holarricine	Seeds	Siddiqui *et al.*	1981
44.	Holacine	Bark	Siddiqui *et al.*	1982
45.	Holacimine	"	-	1982
46.	Regholarrhenine A, B, C	"	Bhutani *et al.*	1988
47.	Holarrifine	"	Siddiqui *et al.*	1989
48.	Regholarrhenine D, E, F	"	Bhutani *et al.*	1990

Fig - 2 STRUCTURES OF ALKALOIDS ISOLATED FROM HOLARRHENA antidysenterica.





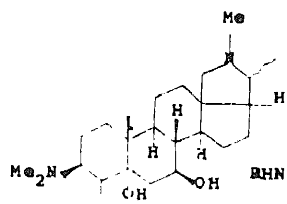
Concuressine



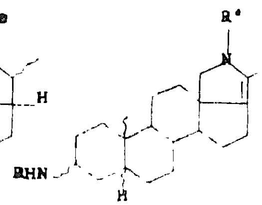
3-Epihetero-conessine.

STRUCTURES OF CONARRHIMINE GROUP OF ALKALOIDS.

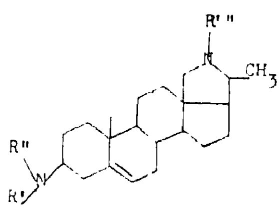
	R'	R"	R'"
Conarrhimine	H	H	H
Conimine	CH_3	H	H
Conessimine	CH_3	CH_3	H
Conamine	H	H	CH_3
Isoconessimine	CH_3	H	CH_3
Conessine	CH_3	CH_3	CH_3

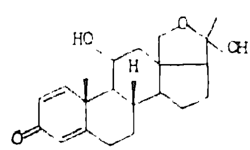


Kurcholessine

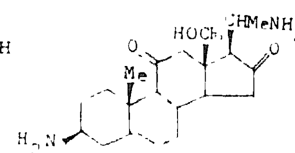


Kurchamine - R=H, R'=Me
Kurchimine - R=Me, R'=H.





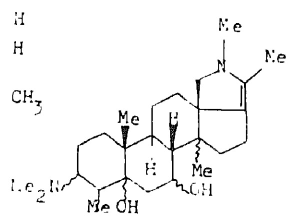
Holadysone.



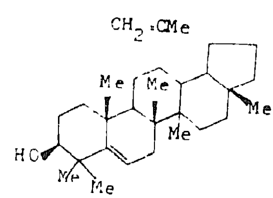
Holarrhenine.

STRUCTURES OF CONKURCHINE GROUP OF ALKALOIDS.

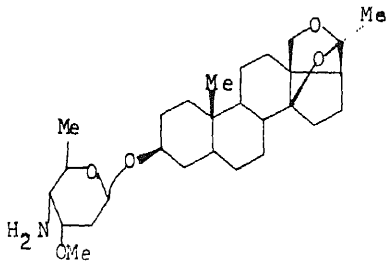
	R'	R"	R'"
Conkurchine	H	H	H
Conessidine	CH_3	H	H
Trimethyl conkurchine	CH_3	CH_3	CH_3



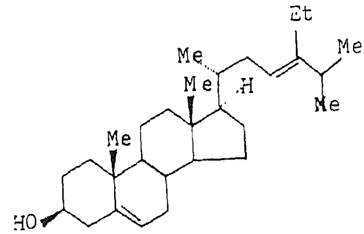
Holacine



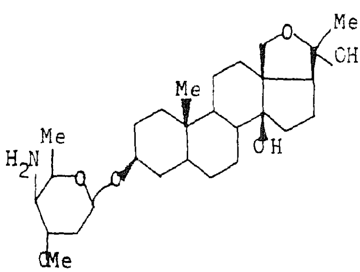
5,20(29)-lupadien-3β-ol.



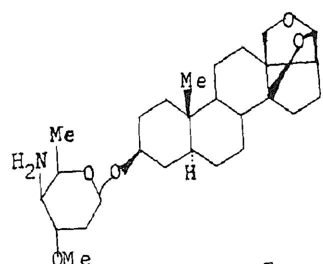
Holarosine B



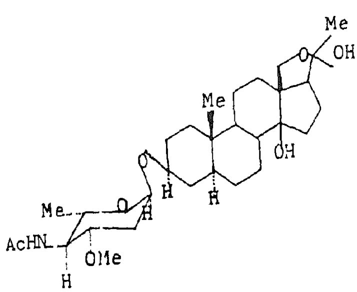
Sitosta-5,23-dien-3β-ol.



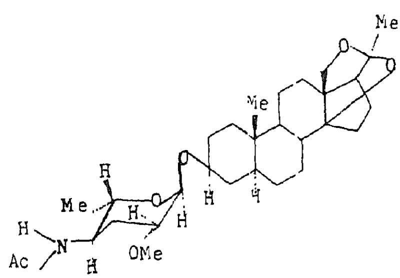
Holantosine E



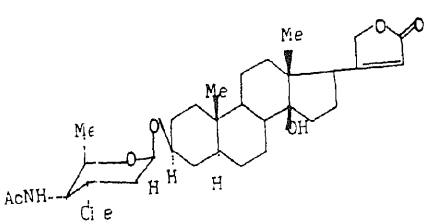
Holantosine F.



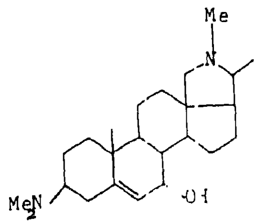
N- Acetyl holantosine C



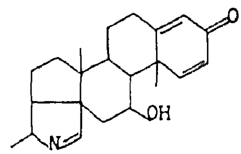
N-Acetyl holantosine D



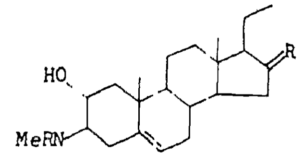
N-Acetyl holarosine.



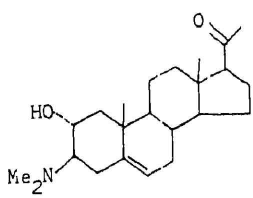
7α-hydroxyconessine



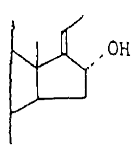
Holanamine



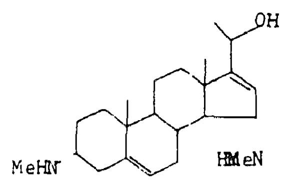
Kurchiphyllamine R=H ; R'=O
Kurchiphylline R=Me ; R'=O



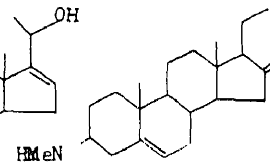
Kurchiline



Kurchaline



Holadysamine.



Holadysine

Siddiqui *et al.*, (1934) studied the action of cyanogen bromide on conessine and its N-demethylation to isoconessimine and conimine. Irani *et al.*, (1946) isolated 1.4% of glyco alkaloid and galactose as one of its hydrolysis product. Alfred Bertho (1951) substantiated the structures of conessine and conkurchine. Haworth *et al.*, (1953) studied the position of the double bond and the dimethyl amino group of conessine. Ganguly *et al.*, (1953) isolated 0.01% of $\beta_{2\,sterol}$ ($C_{29}H_{50}O$) similar to β sitosterol from the unsaponified fraction of the Kurchi bark. Alyn (1957) reported the synthesis of methyl benzo (C) phenanthrenes - alkaloids of kurchi. Ram *et al.*, (1962) studied the action of nitrous acid on holarrhimine. Bhattacharya *et al.*, (1962) studied the synthesis of conessimine from conessine.

Rudolf *et al.*, (1963) isolated holadysone-11a-20-dihydroxy- 18- 20-epoxy pregna-1-4-dien-3-one, and glycosides, stigmastadienol, stigmastenol and ergostenol from Kurchi bark. Mansa and Bhattacharya (1964) studied the structural correlation of holarrhimine and paravallarines. Godse *et al.*, (1963) studied the effect of neighbouring groups in derivatives of holarrhimine. Victor (1964) detected the presence of L- quebrachitol in Kurchi. Labler *et al.*, (1966) isolated some secondary formed weak bases like (20-R)-3a-(dimethyl amino)-18, 20-oxidopregn-5-ene, (20R)- 3β-(dimethyl amino)18-20-oxidopregn-5-ene, 3β-(dimethyl amino) pregn-5-ene-20-one and Carbonyl- N,-N-bis (3β-dimethyl amine-N-dimethyl Conan-5-ene), from Kurchi.

Other Constituents of Kurchi

Number of constituents other than alkaloids have been reported in the various parts of Kurchi. Powell *et al.*, (1969) isolated 9-D- hydroxy-*cis*-12-octa decenoic acid from the seed oil of kurchi. The known occurrence of this acid was previously limited to the genus strophanthus. Bhattacharjee *et al.*, (1969) and Kapoor *et al.*, (1969) reported the presence of terpenes and alkaloids and the absence of sterols, saponins, tannins and flavonoids in the stem bark of kurchi. Goutarel (1970) reviewed the new type of gluco alkaloids, the aminoglucosteroids, isolated from the Asiatic species of the family Apocynaceae. Daniel *et al.*, (1978) detected 2.3% of tannins in the leaves of kurchi.

Thanki and Thaker (1950) isolated about 15 amino acids from the seeds of Kurchi, the dominant amongst them being aspartic acid and argenine. The amino acid content of the protein hydrolysate of the seed was found to be comparable with that of groundnut seeds. Narayana and Naik (1981) isolated a triterpene, 5,20 (29)-Lupadien-3β-ol, a first natural product known with a double bond in ring B of the lupane skeleton from the bark of Kurchi. They also isolated Sitosta-5, 23, dien-3β-ol from the bark. Singh (1983) studied the leaf protein of kurchi. The Wealth of India (1959) mentioned the composition of certain constituents of Kurchi like gum, seed oil, latex, etc, and described their standards.

Analytical profiles

Schroff and Dhir (1939) developed an assay for Kurchi and Kurchi bismuth iodide. Karkun and Goha (1943) proposed a method of analysis for Kurchi alkaloids which later on, was included in IPL (1946). Basu and Mithal (1947) investigated the thermolabile and alkali unstable conditions of Kurchi alkaloids and indicated the defects of IPL method which mentioned prolonged heating and association of alkaloids with alkali. Avoiding these conditions they suggested a better method for the assay of Kurchi alkaloids using ethanol : chloroform (1 : 3), containing 2% ammonia as solvent for extraction at 50°C (Basu and Mithal 1948). Rao (1948); Basu and Bhattacharya (1949) proposed volumetric methods for the estimation of Kurchi alkaloids. All these methods later on were comparatively studied by Dutta and Ghosh (1949). Piette (1949) validated the method of Mascre and Loiseau (1941) and used it for the estimation of connesine in pure solution. Ommen (1951) reported 43% of thermostable and 57% thermolabile types of alkaloids in Kurchi. Jaishanker and Basu (1961) and Labler and Cerny (1963) separated Kurchi alkaloids by paper chromatography and thin layer chromatography respectively. Vishin and Gupta (1967) estimated the alkaloids of Kurchi bark by nonaqueous titrimetry. Jayaswal and Basu (1967) estimated conessine spectrophotometrically in Kurchi and *W. tomentosa* barks. Khorana and Vasudevan (1967) devised a method for the estimation of conessine in the formulations of kurchi bark. Dwivedi and Sharma (1990) developed a turbidimetric method for the quantitative estimation of the total

alkaloids of kurchi bark in crude medicinal preparations and in the body fluids of man and rat.

Some of the other investigations reported for kurchi are as follows:

Gupta and Sen Gupta (1946) utilized diastase in the extraction of alkaloids and obtained an increased percentage of alkaloids from 0.6 to 1.18% in the chloroform extract of Kurchi bark. The degrading action of diastase was specific on cellular matter, and made the cell wall more permeable to organic solvents. Thakkar *et al.*, (1972) employed ultrasonic energy for the extraction of the alkaloids from kurchi bark.

Sharma and Bal (1959) studied the effect of the extract of kurchi bark on plant tissue. He exposed the root tips of onions (*A. cepa*) to dilute solutions of the bark extracts for different periods of time and observed the immediate and delayed effects. The bark extract was found to be very toxic and it caused pycnosis and agglutination of the chromatin substance, without formation of tumours.

Daniel *et al.*, (1978a) studied the chemotaxonomy of Apocynaceae. Chary *et al.*, (1983) screened Kurchi for its protease activity. Roy *et al.*, (1988) reported the presence of storage fungi *Aspergillus flavus* in Kurchi and studied their mycotoxins (1990). Shyam (1989) studied the copper accumulating ability of Kurchi.

Pharmacological and Clinical Studies

Amoebicidal and other activities of Kurchi and its individual alkaloids, especially conessine have been extensively investigated by number of workers.

Brown (1922) reported very good antidiarrhoeal effect of the seed extracts of Apocynaceae plants containing conessine, in cases of chronic amoebic dysentery

Chopra *et al.*, (1927) have reported number of pharmacological actions of conessine, like its feeble toxicity on protozoal flagellates such as *Trichomonas hominis*, inhibition on the activity of digestive enzymes such as pepsin and trypsin, cardiac irregularities in large doses and its specific toxic effect on *Entamoeba histolytica*. The toxicity of conessine, holarrhine and oxyconessine was exhibited *in vivo* on Tubercle bacilli (Meissner 1930). Chopra *et al.*, (1933) studied the pharmacological action of Kurchicine and found it to be a protoplasmic poison like emetine. The alkaloid stimulated the plain muscles of the intestine and uterus, dilated the vessels of the splenic area, produced a fall in blood pressure and has a direct depressant action on the heart, in particular the auricular ventricular Bundle.

Bakhsh (1936) determined the lethal doses of conessine, kurchicine and isoconessine and studied the various pharmacological actions of these alkaloids. Conessine raised (in small doses), and lowered (in large doses) the B.P., contracted the renal vessels, dilated the intestinal vessels but did not have any effect on the coronary vessels of isolated rabbit heart. Isoconessine was less toxic in comparison to conessine and showed a more marked stimulating effect on frogs voluntary and

smooth muscles of intestine and uterus. Siddiqui (1936) carried out a comparative pharmacological study of conessine, isoconessine and neoconessine.

Alfred (1944) showed that conessine, conessidine, conkurchine, kurchicine and holarrhenine in high dilutions, kill paramecia, colpidia and daphnia, like emetine. Jones (1947) and Piette *et al.*, (1949) compared the amoebicidal properties' of conessine and emetine and indicated that the amoebicidal action of conessine was superior to emetine *in vivo*. Their clinical studies further indicated that even though conessine is a more satisfactory drug in cases of acute intestinal amoebiasis associated with secondary infection or for patient refractory to emetine, it is not effective like emetine in cases of hepatic or cystic amoebiasis.

Lavier *et al.*, (1948) studied the antiamoebic activity of conessine and showed it to possess good results with negligible side effects like trembling, nightmares or insomnia.

Duriex *et al.*, (1948) reported spectacular antidiarrhoeal activity of conessine in cases of primary and secondary infections where emetine failed to work.

Piette (1950) found the lethal doses of conessine hydrochloride (1 : 40,000 to 70,000) and emetine hydrochloride (1 : 2890 to 4800) for actively growing amoebae.

Piette (1950) and Auffret (1950) studied the accumulating property of conessine in different organs of experimental animals. Pluchon (1950) stated that sub-therapeutic doses of conessine are also prone to get fixed in various organs like spleen, lungs, liver, kidney and brain and could be detected weeks later.

Lambir, Bernard (1953), and Mukerji (1953) observed in vitro,, the inhibiting action of conessine on the growth of *Mycobacterium tuberculosis*.

The Wealth of India (1959) mentions good results of a glycerine suppository containing conessine hydro bromide in cases of trichomoniasis.

Jaishanker *et al.*, (1961) studied the general pharmacological actions of conkurchine hydrochloride and reported it to be devoid of any significant effect on the isolated rectus abdominis of frog, ileum, uterus and CNS of rat, but decreased the heart rate of frog, lowered the blood pressure of the dog and dilated the blood vessels of rat in higher doses. Bhavsar *et al.*, (1965) studied the fresh juice of kurchi leaves for its bacteriostatic activity against *Staph. aureus* and *E. coli* and found it to be ineffective. Basu and Jayswal (1968) tested conessine *in vitro*, against the "C" strain of *Entamoeba histolytica* and reported it to be a more potent amoebicidal agent in comparison to conessine dihydrate, conkurchine, holarrhine and kurchinine.

Dhar *et al.*, (1968) studied the pharmacological activities of the alcoholic extract of stem, fruits and stem bark and reported the former two to possess antispasmodic activity on isolated guinea pigs ileum but did not notice antibacterial and antifungal activity and gross behavioral effect in mice. Stem bark (L.D50 1000

mg/kg orally in mice) was hypotensive without antiprotozoal, anticancer and hypoglycemic activities while the fruit ($L.D_{50}$ 250 mg/kg i.p in mice) was antiprotozoal, anticancer, hypoglycemic and antispirochetal. None of the extracts exerted any antiviral or anthelmintic activities against certain selected viruses and intestinal parasites respectively.

Singh and Singh (1972) found that the bark extract increased the lesion number against potato virus x(PVX). Raj (1974) showed that the stem bark extract did not have any effect on human *Ascaris lumbricoides in vitro*,.

Nandi and Mazumdar (1979) reported the maximum antispasmodic activity of the homeopathic tincture of kurchi bark prepared by using 70% alcohol.

Deshmukh and Jain (1981) mentioned that the seed oil of kurchi showed an inhibitory effect against pathogenic keratinophillic fungi, like *Chrysosporium indicum, C. pannicola, Malbroanchea aurentiaca, Keratinomyces ajelloi* and *Microsporum gypseum*.

Clinically, polyherbal Ayurvedic formulations containing stem bark of kurchi as one of the ingredient possessed good antigiardiastic, (Singh and Chaturvedi 1981, 1982a) and antidysentric and antidiarrhoeal properties (Javalgekar 1982).

Chaturvedi and Singh (1983) conducted a study on the side effects of kurchi bark powder in 11 indoor patients and observed that the drug can lead to subjective symptoms as well as to hypotension. Abrol and Chopra (1965) reported the negligible insecticidal activity of the alcoholic extract of the bark against house flies and mosquitoes. Suryakala *et al.*, (1983) have studied the juvenomimetic activity of the extract of stem on *Dysdercus similis, Spodoptera litura, Musca domestica* and *Anopheles stephensi* and showed it to possess a gonadotropic effect on the females, except in *A. Stephensi*.

Thappa *et al.*, (1989) have observed a wide range of insecticidal activity of conessine with 0.5 to 10ppm dose against *Aedes aegypti, Dysdercus koenigii, Spodoptera litura* and *Picris brassicae* species.

The authors personally communicated with the tribal people of Gujarat residing around Surat and Junagadh districts and found them to be using the decoction of the seeds of kurchi in cases of diabetes. The review of the literature did not indicate the use of the seeds in cases of diabetes mellitus, but certain Ayurvedic antidiabetic formulations like Nyagrodhadi Churna (Swami 1950), Asanadi Ghanvati (Pandya 1991), Phaki (Ainapure *et al.*, 1985), etc. incorporating the seeds of kurchi are known. Hence, the authors undertook screening of seeds of kurchi for their hypoglycaemic and antidiabetic activity.

The aqueous and alcoholic extracts (95% ethanolic) of the drug at a dose of 250 mg/kg body weight P.O. were tested for their effect on the blood sugar level on albino rats by normal fasted model and Glucose loaded model. Both the extracts exhibited significant hypoglycaemic effect in both the models. The extracts were then tested on streptozotocin induced hyperglycaemic rats at the same dose level

and was found to produce significant hypoglycaemic activity (Gopal and Chauhan 1993).

The seed and bark of kurchi contains almost the same chemical constituents, but seeds alone have been proved to possess hypoglycaemic activity and not the bark (Dhar *et al.*, 1968). These observations suggested the possibility of some constituents other than alkaloids responsible for the hypoglycaemic property of the seeds of Kurchi. Proteins like insulin and polypeptide P (Khanna *et al.*, 1981) have been proved to possess good hypoglycaemic activities. Hence the hypoglycaemic properties of primary metabolites was thought worth to investigate. The protein fractions of kurchi seeds at a dose of 100 mg/kg body weight i.p. in normal fasted model and glucose loaded model of albino rats did not reveal significant hypoglycaemic activity (Trivedi *et al.*, 1991). The hypoglycaemic activity of the other fractions of the seed are under investigation.

Tissue Culture

Considerable work has been done on the tissue culture of kurchi with its seedlings as the explant.

Heble *et al.*, (1971) isolated 24-methylene cholesterol from callus tissues raised from germinated seedlings of kurchi and established callus tissues and various cell lines. Heble *et al.*, (1973) studied the effect of various phytohormones such as IAA, NAA and cytokinins on the growth and production of secondary constituents of callus tissues, and noticed significant accumulation of the metabolites of cholesterol like 24 -methylene cholesterol and 28-isofurastane and the inhibition of conessine synthesis in the callus tissues of kurchi. Based on these facts they have suggested that a modification of steroid metabolism under cultural conditions is possible (Heble *et al.*, 1974). When cholesterol-4-C^{14} was administered to 10 days old callus, radioactive 24-methylene cholesterol, 28-isofucosterol, sitosterol, stigmasterol and conessine, were produced, there by indicating the conversion of cholesterol into sitosterol mediating through 24-methylene cholesterol and 28 isofucosterol in this system (Heble *et al.*, 1976a). Callus cultures derived from the hypocotyl of germinated seedlings of kurchi showed an inherent lack of organ forming ability when grown under the influence of a wide range of exogenous growth factors. A number of sterols were isolated from the callus, of which the predominant ones were identified as cholesterol, 24 - methylene cholesterol, 28 - isofucosterol, sitosterol and stigmasterol (Heble *et al.*, 1976). Several sterols and steroidal alkaloids were detected by them in the suspension culture of kurchi (Heble *et al.*, 1977).

Panda *et al.*, (1991) established callus and suspension cultures of kurchi for the production of steroidal alkaloids especially conessine. The doubling time and specific growth rate of cells in suspension culture were computed to be 47.5 hr. and 0.35 hr per day respectively. A maximum of 300 mg alkaloid per 100 g dry cell wt

in 40 days and 130 mg per 100 g dry cell wt in 8 days were obtained in the callus and suspension cultures respectively. About 90% of the total alkaloids produced in the cell culture was conessine. They also studied the effect of the major nutrients on the growth and alkaloid production. A modified Murashige and Skoog (MS) medium that contains 60 mm total N with a NH^{+}_{4} to NO^{-}_{3} ratio of 5:1, 0.25 mm phosphate and 4g/L sucrose was developed for increasing the yield of conessine. The growth regulators 2,4- D and kinetin were found to affect the alkaloid synthesis. Using an optimal level of inoculum (3g/L). The modified medium resulted in alkaloid synthesis of 0.66 g/100g dry cell wt; which represented a 4.25 fold increase over that obtained in standard M.S. medium (Panda *et al.*, 1992). A precursor feeding strategy for increasing the yield of conessine in cell suspension culture was also established by them. A total of 50 mg/L of added cholesterol was converted to 43 mg/L of alkaloids 90% of which was conessine. By applying the precursor feeding policy to the cell suspension culture in modified Morashige and Skoog (MS) medium a total of 143 mg/L of alkaloid was produced in 8 days. In this way the alkaloid content of the cells were increased 76 fold compared to that obtained in the standard MS medium. The steps leading to biotransformation of cholesterol to alkaloids were unaffected by phosphate. The shake flask data were successfully transferred to a bench scale 6 L stirred tank bioreactor in which the biosynthetic rate of alkaloid production was 110 mg/100 g dry cells 160 fold higher than that of whole plant (Panda *et al.*, 1992a).

Rajashekar *et al.*, (1973) have established a method for isolating the protoplast from the cultured plant cells of kurchi. Various factors affecting the release of protoplast from the cells like effect of pectinase, age of cells, effect of organic and inorganic sodium salts etc. were studied. Further they have reported morphological observations of the isolated protoplasts, also.

Dohnal Barbara *et al.*, (1990) studied one and six year old callus tissue of .kurchi and reported five alkaloids, two of them being as conessine and conimine. The alkaloidal extract was found to inhibit the growth of *Shigella sonnei, Sh. flexneri* and *Salmonella enteritidus* strains but not of *S. typhi* and *S. paratyphi*.

Kulkarni *et al.*, (1992) have worked on the *in vitro*, propagation of kurchi. They found IAA (2mg/L) to be the most favourable, for inducing the callus in root and stem and 2,4-D (0.5 mg/L) in leaf explants. The explants taken from the nodal segments of stem regenerated to shoots on MS medium supplemented with IAA (1mg/L) and on transferring it to medium containing 3mg/L of IAA, the shoots developed roots leading to formation of complete plantlets.

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Studies on Commercial Indian Aconites : Atis

S. K. Kapur

Regional Research Laboratory,
Canal Road,
Jammu Tawi - 180001, India

Introduction

Atis roots constitute one of the major items of crude drug trade in the country and are extensively used in indigenous system of medicines. Inspite of a good amount of work on the taxonomy and identity of the drug as well as the whole plant a great amount of confusion still persists. Studies revealed that neither there are proper estimates of the raw material nor there are any standards for the drug. Similarly the drug was found to be much confused by a number of misnomers and is frequently adulterated by other cheaper roots. A detailed investigation on the plant, the root drug and its trade revealed a number of facts and the same are being discussed in the paper.

Pharmacognostic studies of the roots of *Aconitum heterophyllum* have previously been done by Datta, *et al.*, (1945). But no work on adulterants or anatomical comparison of different plants has been done. The genus *Aconitum* has been subjected to a great taxonomical confusion. Stapf (1905) investigated in detail the Indian species of *Aconites* and separated them into 3 groups viz., Napellus type, Anthora type and Deinorrhizum type. *Aconitum* species yielding the non poisonous Aconite roots are inlcuded under Anthora type. His classification is based on purely anatomical characters of the root.

Uses and Chemical Constituents

In the indigenous system of medicine the drug is considered a valuable febrifuge and a bitter tonic especially in combating debility after malaria or other fevers. The drug in combination with certain other ingredients is also used as antiperiodic The roots have been reported to contain the alkaloids viz., Atisin, hetratisine, hetisine and heterophyllin Bose (1932), all of which are non-toxic. Almost whole of the produce is consumed by manufactures of Ayurvedic medicines, Vaids and Hakims. Due to the great demand of drug it is sold in the market at a very high price.

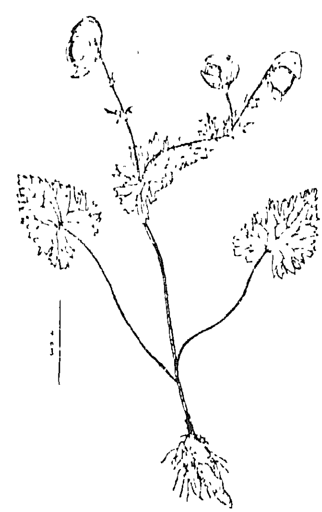
Plant Description, Occurrence and Distribution

Atis of commerce is mainly constituted by the roots of a perennial herb *Aconitum heterophyllum* Wall. The roots of yet another species *Aconitum kashmericum* Stapf ex Coventry a closely allied species are also harvested as Atis are sold in the market as frequent adulterant or as a cheap substitute by the name of Atis Kashmiri or Kala Atis or *Aconitum spicatum*.

Aconitum heterophyllum : It is an erect, herb, reaching upto 75 cm. in height with purple marked pale green flowers in terminal racemes which frequently branch to a panicle, the lower leaves are long petioled while the upper are stem clasping and acute (Fig. 1).

Aconitum Kashmericum: Seldom reaches a height of more than 30 cm. and has deep blue flowers. Leaves few, long, petioled, orbicular, cordate to ovate cordate, incised and toothed (Fig.2).

Sketch diagram of a plant of *Aconitum heterophyllum*



Sketch diagram of a plant of *Aconitum kashmericum*

Aconitum heterophyllum occurs mostly under the 'banks and mixed conferous forests and also on the moist banks of nallahas at about 3000 m. and extends well above the tree limit beyond 4000 m. On the other hand *Aconitum kashmericum* is exclusively found among the alpine meadows and boulders where sometimes dense formations are noticed. The two simultaneously flower during the pre-autumn months from August to first week of October.

Aconitum Kashmericum is mainly distributed among the alpine localities of Kashmir, Jammu, Chamba and Kinnaur Himalayas, while the former grows extensively throughout N.W. Himalayas extending from Jammu and Kashmir and in the Western part of Nepal. In *Aconitum kashmericum* the upper sepals of the flowers form a hood over the other sepals, sub-orbicular carpels with long yellowish hairs, limb of nectary narrowed to a point at the top. In *Aconitum heterophyllum* sepals 5, petal like, erect, the upper one (helmet) pointed in front, top rounded, lateral sepals as long as the helmet. In form of leaf it varies considerably (Watt., 1972).

The Drug

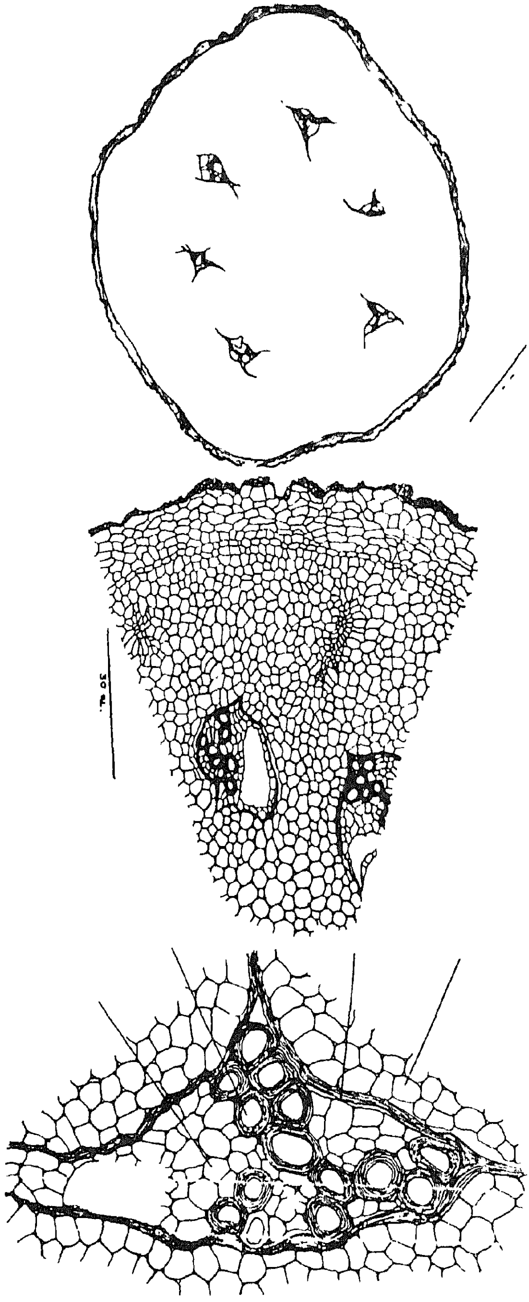
The fresh fully grown roots derived from *Aconitum heterophyllum* is of ash grey colour about 1-15 inch and about 0.5 inch thick at its upper edge. Roots of good quality break with a short starchy fracture presenting a uniform milky white surface. The taste is purely bitter and no tingling sensation is produced. The roots derived from *Aconitum kashmericum* on the other hand are smaller, darker in colour and between 5-8 mm. in thickness at its upper edge. The tuber is white, inside fracture is similar to that in *Aconitum heterophyllum*. These are less shrivelled and have rounded root scars, in orbicular rings, while in the case of *Aconitum heterophyllum* the root scars are swollen and longitudinally extended.

Roots of *A. heterophyllum* are biennial and paired, cylinderic oblong or slightly conic external surface. The buds at the crown are short, 0.3-0.8 cm. long.

Pharmacognostic Studies

Microscopic characteristics

A T. S of *A. heterophyllum* shows 3-5 layered epidermis or metaderm of submerged elongated cells which are generally crushed in the old roots. Bark yellow to orange colour. Primary cortex in young roots is 5-6 layered with oval or radially elongated cells (Fig.3a- c). The primary cortex is separated by a unicellular endodermis composed of elongated spindle shaped cells from the inner bark which is a uniform tissue having cells full of starch grains. Secondary phloem elements are scattered in this tissue both inside as well as outside the cambium strands. The cambium is broken into 6 triangular steles arranged in the form of a concenteric ring. Thus, the whole of central cylinder practically consists of secondary phloem plus a narrow pith whose cells are circular or oval in outline and have got starch grains. The cambium of the isolated xylem strands or the scattered "steles" is very delicate and



a. T.S. root of *A.heterophyllum* showing 6 vascular bundles

b. T.S. of a mature root of *A.heterophyllum*

c. Enlarged meristele of *A.heterophyllum*.

usually gets crushed in T. S. Xylem tissue inside it is more or less V-shaped, meeting in the centre with or without cavities formed by the disintegration of the cells of xylem parenchyma.

Measurements of the cells in T.S. are :- parenchyma cells of secondary cortex containing starch α 9-19-27 μ, β 14-27-53 μ endodermis cells α 4-8 μ, β 4-6 μ ; xylem vessels measure of α 41-71-282 μ x β 8-16-21 μ.

T.S. root of *A. kashmericum* shows the metaderm consists of one or more layers of suberised cells with brown or yellowish walls and is often cast off in mature roots. In very young roots primary cortex is clearly observed being composed of 6 or more layers of cells; cells very much elongated, endodermis demarcates between primary and secondary cortex, endodermal cells in T. S. measure α 5.7-9.5-13.3 μ, β 9.5-17-24 μ. Secondary cortex is a uniform tissue of parenchymatous cells and full of starch grains. In this tissue are embbeded 4-5 isolated cylindric strands of xylem or steles arranged in the form of ring, each one having its own circular rings of cambium. Cambium is very well differentiated into 3-4 layers of cells, more or less of isodiametric type. Stone cells as in *A. heterophyllum* are absent in this case. Groups of secondary phloem or sieve tissue are found scattered throughout the central cylinder. Xylem is star like in shape enclosing xylem parenchyma which is unoblitelateral (Fig. 4a,b). Pith is very small practically unidentified.

Parenchyma cells of secondary cortex in T. S. measure α 17-21-36 μ, β 17-27-59-μ; xylum vessels measure α 58-116-191 μ and β 6-8-16 μ. These have got narrow lumen.

Anatomical Characteristics of Powder

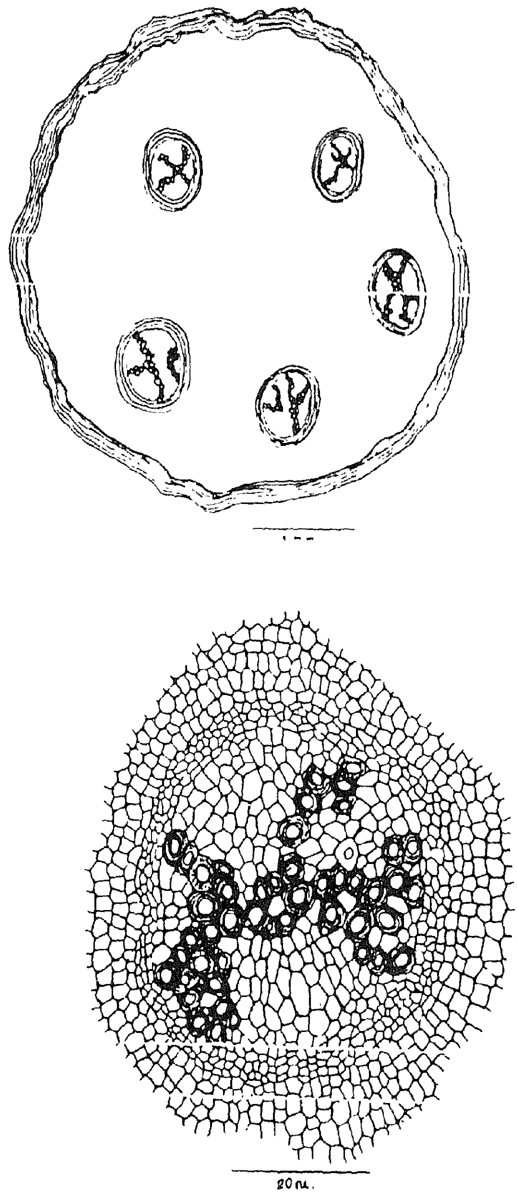
In powder form no apparent distinction between two species is found. Root powder of the two species (90-100 mesh) was studied with a view to find out:

1. Structure of starch grains.
2. Presence or absence of calcium oxalate crystals covering trichomes and
3. Lignified vessels, fibres, tricheids, parenchyma and stone cells.

Under the microscope the powder from the two species show very little or no difference at all. The common features of the two species in powder form are:

1. Abundance of starch grains in both types. They vary in size and are from rounded, oval to lunar in shape with central hilum, found in groups of 2,3,4 or even more.
2. Fragments of xylem vessels, masses of starchy parenchyma. Vessels have got spiral, annular and reticulated pittings. Tracheids and fibre tracheids found the latter have got simple pits. Calcium oxalate crystals and epidermal trichomes with phloroglucinal and HCl xylem vessels show little lignification.

The various chemical tests performed with the powder are tabulated in Table 1.



a. T.S. root of *A.kashmericum.*, showing 5 vascular bundles
b. T.S. root of *A.kashmericum*

Table 1. Comparison : T.S. Roots of A.heterophyllum and A.kashmericum.

T. S. Root		A. heterophyllum	A.kashmericum
1.	Primary cortex	10 or more layered	6 layered or less.
2.	Secondary cortex	Composed of starchy parenchyma, starch grains both simple and compound, stone cells absent. Sieve tissue found in patches.	Same
3.	Cambium	Discontinuous, split into isolated strands arranged in the form of a ring, Crushed in T.S.	3-4 layered cambium, very clearly differentiated, discontinuous.
4.	Stele	A ring of upto 6 vascular bundles observed.	4-5 vascular bundles in a ring.
5.	Structure of vascular bundle	Xylem either U or V shaped or tangentially flattened with or without cavities in the tissue.	Xylem is well marked stellate shape with no cavities.
6.	Endodermis	Present one layered	One layered.

Conclusion

Market samples of Atis or Nirbishi consist of not only of a particular species of *Aconitum* but rather a admixture of two or more species of *Aconitum.* They vary considerably microscopically and not macroscopically.

Acknowledgements

The author is thankful to Dr. R. S. Kapil, Director, Regional Research Laboratory, Canal Road, Jammu Tawi for his interest in the present work. Thanks are also due to Dr. B.L.Kaul, Head, Botanical Sciences, R.R.L., Jammu and to Shri Ashok Kumar, Anokh Singh, Dr.Verandra Singh and Shri Kuldip Kumar for necessary help.

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Chemistry and Biological Activity of *Tylophora* species

D.S.Bhakuni and Archna Rani

Central Drug Research Institute,
Lucknow - 226 001.

Introduction

THE GENUS *Tylophora* (Asclepiadaceae) comprises 50 species which are distributed in Africa, Asia, Australia and Oceanic Islands. Some of the *Tylophora* species have reputation in folklore medicine. In the Philippines, the roots of *T. brevipes* are used as a substitute for *Ipecacunha* and the leaves of *T. perroteltiana* for healing wounds[1]. In Taiwan, stem juice of *T. brownii* Hay is used by the natives on wounds[2]. In Malay Peninsula the leaves of *T. tenuis* Bl. are used for treatment of scabies[2]. In Indonesia, the leaves of *T. cissoides* Bl. are applied for releaving abdominal pain whereas in Philippines, decoction of the roots of *T. brevipes* (Turz) Ville is considered to be emetic and carminative[2]. The roots are chewed and swallowed for indigestion. *T. conspicua* and *T. sylvatica* are also used medicinally[3,4].

In India, *T. asthmatica* W. & A. is known as Indian *Ipecacunha* and reported to have emetic, expectorant and antidysenteric properties[6,7]. The plant is a perennial twining climber with long and fleshy roots. Stems are elongate and glabrous but not much branched. Leaves are 5-10 cm by 2.5-5.7 cm, elliptic-oblong and acute at the top. Flowers are in umbel shape, penducle arises between the petioles. It grows wild in forests and in sandy localities in Bengal, Eastern India, Assam, Orissa, Konkan and in Tamil Nadu upto 1000m.

T. tenuis Blume is reported as an antidote to arsenic and snake poison. It is also used as a cure for perspiration, urticaria and small-pox. The plant grows in West Coast and Coast of Tamil Nadu.

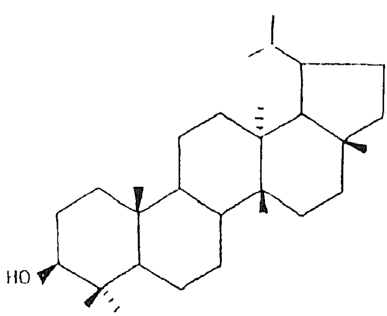
Juice of root of *T. fasciculata* is given with milk as tonic. Leaves are applied to ulcers and wounds to induce healthy granulation. Plant is used as a poison for rats and vermin. It grows in upper Gangetic Plain, eastwards to the Khasia Hills and through Madhya Pradesh and Konkan southwards to Karnataka.

Non-alkaloidal Constituents

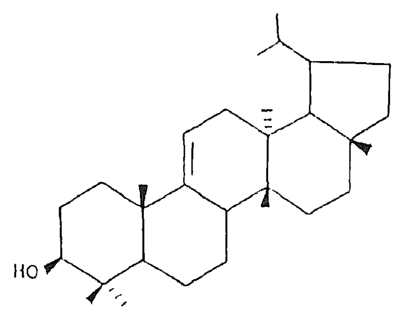
Several *Tylophora* species have been investigated chemically. Both alkaloidal and non-alkaloidal constituents have been isolated and characterized from them.

The root of *T. kerri* collected in Yunnan Lit Cang, China furnished two pentacyclic triterpenes, tylolupenol A, m.p. 184-185° (MeOH); $[\alpha]_D$ + 34.3° ($CHCl_3$) and tylolupenol β., m.p. 177- 178° (MeOH); $[\alpha]_D$- 6.1° ($CHCl_3$), characterized as D : C-friedolup- 8(9)-en-3β-ol (*1*) and D : C-friedolup-9(11)-en-3β-ol (2) respectively[9]. Further paeonal, p-hydroxyacetophenone and β-sitosterol have also been isolated from *T. kerri*[10].

The aerial part of *T. hirsuta* has yielded, octacos-15, 20-dien-11-ol, triacont-15, 19, 23-trien-13-ol, tritriacontane, tritracont-1-ol, gymnorhizol (*3*) m.p 208-210° and β-sitosterol[11,12].



1



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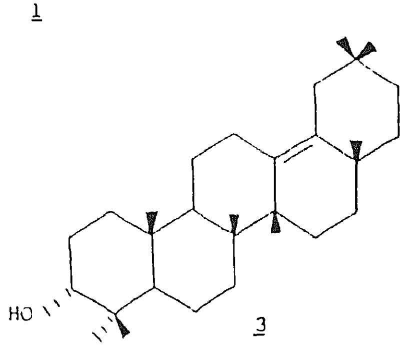


3

Alkaloidal Constituents

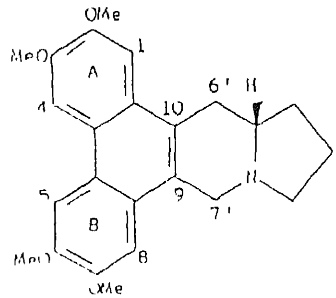
A preliminary survey of some *Tylophora* species revealed the presence of alkaloids in *T. asthmatica, T. crebriflora, T. dalzallii, T. cordifolia, T. flava, T. ovata, T. astrofoliculata, T. mollissima, T. erecta, T. paniculata, T. sylvatica, T. capparidifolia, T. exilis, T. fasciculata, T. goranii, T. hirsuta, T. iphisia, T. longifolia, T. macrantha, T. panciflora, T. rotundifolia, T. tenerrima, T. tenuis*, and *T. zeylanica*. The alkaloids were found to be absent in *T. colorata, T. grandiflora* and *T. conspicua*[13-18].

A number of *Tylophora* species have been extensively investigated for alkaloidal constituents. Phenanthro-indolizidine type of alkaloids have been isolated from *T. asthmatica*[6,19-23], *T. crebriflora*[24,25], *T. dalzallii*[22], *T. ovata*[26], *T. astrofoliculata*[26], and *T. mollissima*[26]. The more common phenanthroindolizidine alkaloids isolated from some *Tylophora* species are tylophorine (*4*), tylophorinine (*5*), tylophorinidine(*6*), and tylocrebrine (*7*). Tylophorine is found the major alkaloid of Indian *T. asthmatica*[19]. Rao *et al.*,[22] isolated three minor alkaloids, alkaloid A, alkaloid B and alkaloid C from *T. asthmatica*. Alkaloid B was identified as O-nortylophorine since it gave tylophorine on O-methylation while alkaloid B was shown to be desmethyltylophorinine. Alkaloid C was found identical with an alkaloid isolated from *T. dalzallii*[22]. Govindachari and his co-workers[23] isolated three minor alkaloids, tylophorinidine (*6*), isotylocrebrine (*9*) and septicine (*8*) from *T. asthmatica* using counter-current technique. Septicine had been isolated earlier from *Ficus septica*[27]. Rao *et al.*,[25] isolated six minor alkaloids from *T. crebriflora* and named them as alkaloid A (*10*), alkaloid *B* (*11*), alkaloid C (*12*), alkaloid D (*13*), alkaloid E (*14*) and alkaloid F (*8*). Alkaloid F was found to be identical with septicine.

Karnich[15] has estimated the total percentage of tylophorine and tylophorinine in different parts of *T. asthmatica* of Sri Lanka in different seasons It was found that alkaloidal contents are comparatively high in flowering period. Phillipson and his co-workers[4] showed by TLC the presence of tylophorinine and three unknown alkaloids in *T. cordifolia* and presence of tylophorine, tylophorinine and four other alkaloids in *T. flava*. These workers have also found that tylophorinine is the major alkaloid of *T. asthmatica* of Srilankan origin whereas tylophorine was the major alkaloid of Indian *T. asthmatica*. Chinese workers have isolated tylophorinine, tylophorinidine and three uncharacterised alkaloids from *T. ovata* and tylophorinine and tylophorinidine from *T. astrofoliculata* and *T. mollissima*[26].

Jackson and co-workers have reported the isolation of two furoquinoline alkaloids, y-fagarine (*15*) and skimmianine (*16*) from the roots and aerial parts of *T. asthmatica*[28]. Govindachari *et al.*,[29] have obtained quaternary alkaloids, dehydrotylophorine (*17*), anhydrodehydrotylophorinine (*18*) and anhydrodehydrotylophorinidine (*19*) from *T. asthmatica*.

Tylophora alkaloids in general are reported to be unstable especially in chloroform solution, in the presence of light and air. Tylophorine is oxidized into



Tylophorine

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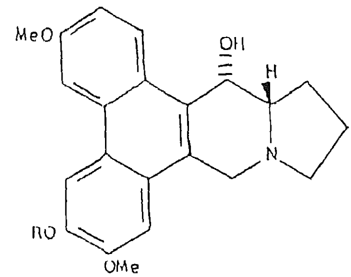
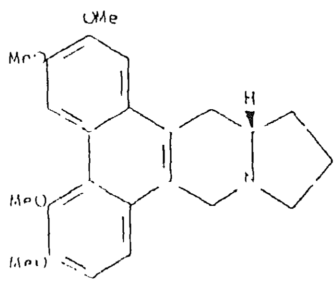
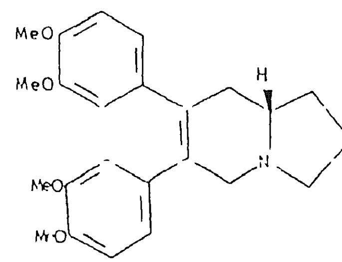


5, R = Me (Tylophorinine)

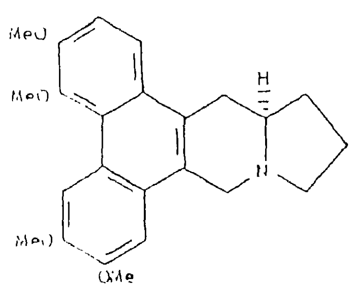
6, R = H (Tylophoridine)



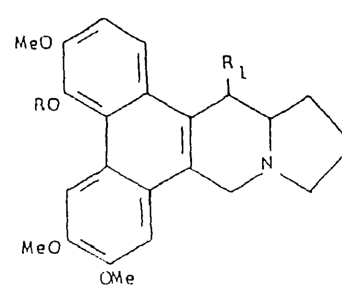
7 Tylocrebrine



8 Septicine



9 Isotylocrebrine



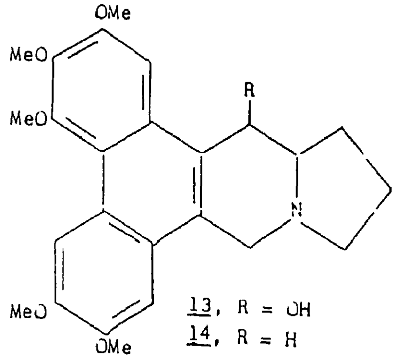
10, R = Me ; R_1 = OH

11, R = R_1 = H

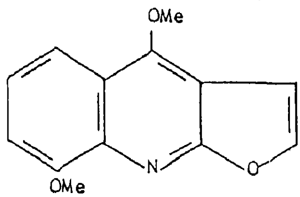
12, R = H ; R_1 = OH

a crystalline product, $C_{24}H_{24}NO_4Cl$. $2H_2O$ (*17*) when kept in chloroform solution at 25° for 26 hr in the presence of light and air, but in the absence of light and air the base is quite stable[29].

The alkaloids isolated so far from *Tylophora* species are recorded in Table 1 alongwith their physical constant and source.



13, R = OH
14, R = H



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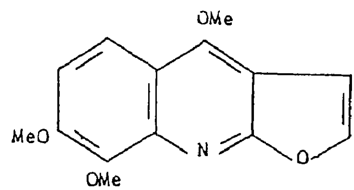

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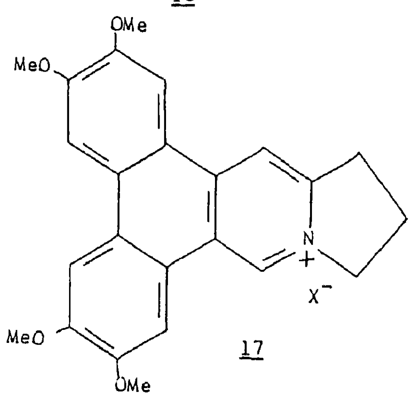

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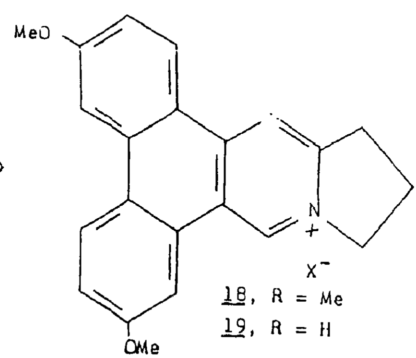


18, R = Me
19, R = H

Chemistry of alkaloids

Most of the *Tylophora* alkaloids have phenanthroindolizidine nucleus. The alkaloids within a group typically differ by the degree of oxygenation on the benzene rings and hydroxylation in the aliphatic moiety. The most important phenanthroindolizidine alkaloid is tylophorine (*4*). The other alkaloids of interest are tylocrebrine (*7*) and tylophorinine (*5*). The chemistry of *Tylophora* alkaloids has been reviewed[30-33]. Short reports on progress in chemistry and on biosynthesis of phenanthroindolizidine alkaloids are also published[34,35].

The spectral properties of the alkaloids is well established and reviewed[36,37]. There is a retro-Diels-Alder reaction, which occurs in the electron impact mass spectrum, but this is diminished in a 14-acetoxy derivative where a McLafferty

Table . 1

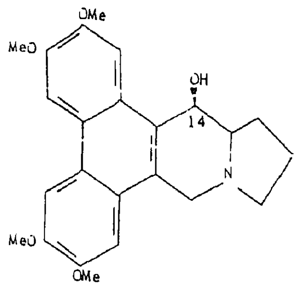
.	Alkaloid	M.P.	$[\alpha]_D$	Isolated from	References
	Tylophorine (*4*)	292° dec.	$[\alpha]^{30}$+ 12.25° (c, 0.733 in $CHCl_3$)	*T. asthmatica* *T. indica* *T. crebriflora* *T. mollissima*	37, 39
	Tylophorinine(*5*)	248-249°	$[\alpha]^{27}$ -14 2° (c, 1.76 in $CHCl_3$)	*T asthmatica* *T. cordifolia* *T. flora* *T ovata* *T. atrafoliculata* *T. mollissima*	14, 19
	Tylophorinidine(*6*)	216-218° dec.	$[\alpha]^{25}$+125 (c, 0.1 in MeOH)	*T. asthmatica* *T indica* *T. atrofoliculata* *T. mollissima* *T. ovata*	
	Tylocrebrine (*7*)	218-220°	$[\alpha]_D$ ^{24}-45° (c, 0.74 in $CHCl_3$)	*T. crebriflora*	24, 44
	Isotylocrebrine(*9*)	212-21°	$[\alpha]_D$+22.43° (c, 1.1 in $CHCl_3$)	*T. asthmatica* *T. crebriflora* *T. hirsuta*	23, 46, 49

Contd.

S. No.	Alkaloid	M.P.	$[\alpha]_D$	Isolated from	References
6.	Septicine(*8*)	136°	$[\alpha]_D$+38.8° (c, 1 in MeOH)	*T. asthmatica*	23
7.	Tylophorinicine(*20*)	210-212° dec.	$[\alpha]_D{}^{25}$-9.4° (c, 0.04 in $CHCl_3$)	*T. asthmatica*	47
8.	Tylohirsuticine(*21*)	200-202°	-	*T. hirsuta*	48, 49
9.	13α-Methyltylo-hirsutinine (*23*)	196- 198°	-	*T. hirsuta*	48, 49.
10.	13α-Methyltylo-hirsutinidine	213- 214°	$[\alpha]_D{}^{25}$+120° (c, 0.6 in $CHCl_3$)	*T. hirsuta*	48, 49
11.	13α-Hydroxy septicine(*25*)	290- 292°	$[\alpha]_D{}^{25}$+100° (c, 0.4 in MeOH)	*T. hirsuta*	48, 49
12.	Tylohirsutinidine (22)	234-237°	-	*T. hirsuta*	48, 49
13.	14-Hydroxyisotylo-crebrine (*27*)	214- 215°	$[\alpha]_D{}^{18}$- 40.29° (c, 0.5 in MeOH)	*T. hirsuta*	49
14.	4-Desmethyliso-tylocrebrine (*30*)	220- 221°	$[\alpha]_D{}^{18}$-50.80 (0.07 in MeOH)	*T. hirsuta*	49
15.	14-Desoxy-13α-methyltylohirsut-inidine (*32*)	180-182°	$[\alpha]_D{}^{18}$+14.66 (0.02 in MeOH)	*T. hirsuta*	49

Contd.

S. No.	Alkaloid	M.P.	$[\alpha]_D$	Isolated from	References
16.	5-Hydroxy-O-methyltylophorinidine(*31*)	245- 247°	$[\alpha]_D^{18}$ +58.97 (c 0.8 in MeOH)	*T. hirsuta*	49
17.	Tylohirsuticine (*33*)	235-237°	-	*T. hirsuta*	49
18.	Alihirsutine A (*34*)	296-298°	-	*T. hirsuta*	50
19.	Tyloindane (*35*)	278-279° dec.	$[\alpha]_D^{30}$ -0.5° (c, 0.2 in AcOH)	*T. indica*	51
20.	Tyloindicine A (*36*)	199-201°	$[\alpha]_D^{35}$ +7.2 (c, 2.1 in MeOH)	*T. indica*	51
21.	Tyloindicine D	229-231°	$[\alpha]_D^{35}$ +1.6 (c, 0.6 in MeOH)	*T. indica*	51
22.	Tyloindicine F (*38*)	245-247° dec.	$[\alpha]_D^{30}$ -0.5° (c, 0.05 in AcOH)	*T. indica*	51
23.	Tyloindicine G (*39*)	235-238° dec.	$[\alpha]_D^{30}$ -2.35° (0.47 in AcOH)	*T. indica*	51
24.	Tyloindicine H (*40*)	225-226° dec.	$[\alpha]_D^{30}$ -5.5° (c, 0.55 in AcOH)	*T. indica*	51
25.	Tyloindicine I (*41*)	215-217° dec.	$[\alpha]_D^{30}$ -2.2° (c, 0.44 in AcOH)	*T. indica*	51
26.	Tyloindicine J (*42*)	180-181°dec.	$[\alpha]_D^{30}$ -2.85° (c, 0.57 in AcOH)	*T. indica*	51



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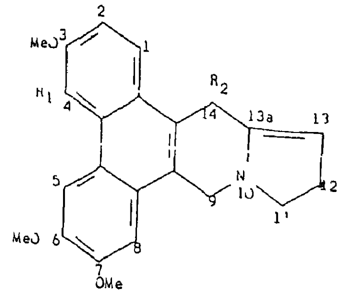
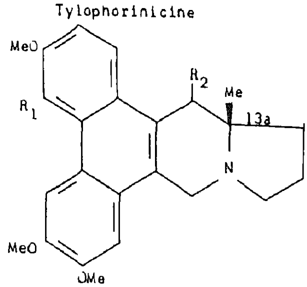
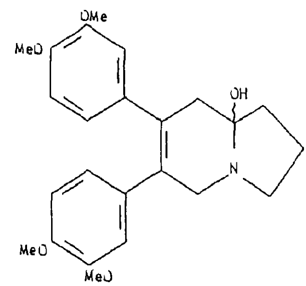


21, $R = OMe$; $R_2 = H$
22, $R_1 = R_2 = OH$

Tylophorinicine



23, $R_1 = OMe$; $R_2 = H$
24, $R_1 = R_2 = OH$



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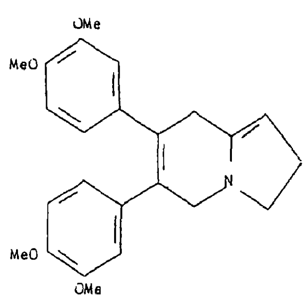
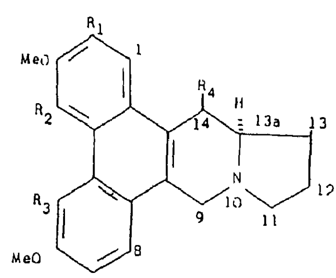




27, $R^1 = R^3 = H$; $R^2 = OMe$; $R^4 = OH$
28, $R^1 = R^3 = R^4 = H$; $R^2 = OMe$
29, $R^1 = OMe$; $R^2 = R^3 = R^4 = H$
30, $R^1 = R^3 = R^4 = H$; $R^2 = OH$
31, $R^1 = R^2 = H$; $R^3 = R^4 = O$

rearrangement predominates The ^{1}H-NMR spectral data for tylophorine are not well known and the ^{13}C-NMR data do not appear to have been established. The absolute configuration was determined by Gellert *et al.* [24].

Several synthesis of *Tylophora* alkaloids particularly that of tylophorine (*4*), tylophorinine (*5*), and tylocrebrine (*7*) are reported. Govindachari and co-workers[30] were the first to report a synthesis of phenanthroindolizidine nucleus. Synthesis of *Tylophora* alkaloids have been reviewed[36,37]. The important *Tylophora* alkaloids are:

Tylophorine

Characterized as 9, 11, 12, 13, 13a, 14-hexahydro-2,3,6,7-tetramethoxydibenzo [f,h] pyrrolo [1,2,-b] isoquinoline.

(S)-Tylophorine (4).- Isolated from *T asthmatica, T. indica, T. flava*[37] short enantiospecific synthesis of (S) (+) - tylophorine is recently reported[39], m.p. 286-287° dec. ($CHCl_3$/EtOH): $[\alpha]_D^{27}$ - 11.6° (c, 1.07 in $CHCl_3$). *B.HCl*: m.p. 276-278° dec. (EtOH aq.) *B HBr*: m.p. 252-255° dec. *B. HNO_3* : m.p. 265-267° (H_2O). *B. MeI* : m.p 280° (H_2O).

N-oxide: pale yellow, m.p. 230° (MeOH)

(R)-Tylophorine. - Obtained by resolution, m.p. 292° dec.; $[\alpha]^{30}_D$ + 12.25° (c, 0 733 in $CHCl_3$).

(+)-Tylophorine:- Synthesized several time. Synthesis reviewed in 1985 [37]. Two new syntheses have been reported since then [39,40].

Tylophorine has powerful vesicant property.

Tylophorinine

It has been characterized as 9,11,12,13,13a, 14-hexahydro-3,6,7- trimethoxy-dibenzo [f,h] pyrrolo [1,2-b] isoquinolin-14-ol.

(-)-Tylophorinine (5):- Isolated from *T. asthmatica*[19], *T. cordifolia*[14], *T. flava*[14], *T. atrafoliculata*[14] and *T. mollissima*[41], m.p. 248-248° (233-235° dec.) ($CHCl_3$/MeOH); $[\alpha]^{27}_D$ -14.2° (c, 1.76 in $CHCl_3$) largely racemic. *B. HCl* m p 257° dec (EtOH). *B. HNO_3* : m.p. 240- 242° (H_2O). *B. acetate*: needles (C_6H_6/Pet. ether or MeOH) m p 222-223° (177-178°)

(+)-Tylophorinine Isolated from *Ficus hispida* (Moraceae) crystallised from $CHCl_3$/EtOH or $CH_{26}Cl_2$/Et_2,^{O}, m.p. 215- 216° dec., 229-231° dec.; $[\alpha]_D$ + 116° (c, 0.26 in $CHCl_3$), $[\alpha]_D$+161°(c,1 5 in $CHCl_3$)[14]

(±)-Tylophorinine: Obtained synthetically Several syntheses are reported[41], m p 249-252° dec.

Tylophorinidine (*6*):- Has been characterized as 9,11,12,13,13a, 14-hydroxy-3,7-dimethoxydibenzo[f,h] pyrrolo [1,2-b] isoquinoline-6,14-diol. The base (*6*) has been isolated from *T. asthmatica, T. indica, T. atrafoliculata, T. mollissima, T.*

ovata and *Persularia pallida*[42], m.p. 216-218° [CH_2Cl_2/MeOH]; $[\alpha]_D$ + 105° (c, 1.1 in $CHCl_3$); $[\alpha]_D^{25}$ + 125° (c, 0.1 in MeOH). *Di-acetate* : m.p. 193-195° dec. (CH_2Cl_2/MeOH). 6-Methyl ether of base is identical with *tylophorinine* (*5*).

5-Hydroxy-O-methyltylophorinidine, m.p. 245-247°; $[\alpha]_D$ + 48.97° (c, 0.8 in MeOH) is found identical with a minor alkaloid from *T. hirsuta*[43].

Tylocrebrine.- Has been characterized as 9,11,12,13,13a, 14- hexahydro-2,3,5,6-tetramethoxydibenzo [f,h] pyrrolo [1,2-b] isoquinoline[42].

(S)-Tylocrebrine (*7*).-Isolated from *T. crebriflora*[24] pale-yellow, m.p. 218-220° dec. ; $[\alpha]_D^{24}$ - 45° (c, 0.74 in $CHCl_3$). *B.HI*: yellow cryst. (MeOH aq.) m.p. 214-217° dec. *B.HCLO₄*: M.P. 262- 264° dec. (Me_2CO aq.). *B. Picrate*: m.p. 134-136° (Me_2CO/MeOH).

(R)-Tylocrebrine.-Isolated from *Ficus septica*[44], pale yellow crystalline, m.p. 220-222° (MeOH); $[\alpha]^{22}_D$ + 20.5° (c, 0.8 in $CHCl_3$).

(+)-*Tylocrebrine*.- Obtained synthetically, as needles[45] ($CHCl_3$/MeOH) m.p. 219-221°.

Isotylocrebrine (5).-Minor alkaloid from the leaves of *T. asthmatica*, m.p. 212-214° (CH_2Cl_2/MeOH); $[\alpha]_D$+22.43° (c, 1.1 in $CHCl_3$)[23,46]. Synthesis of isotylocrebrine is reported[24].

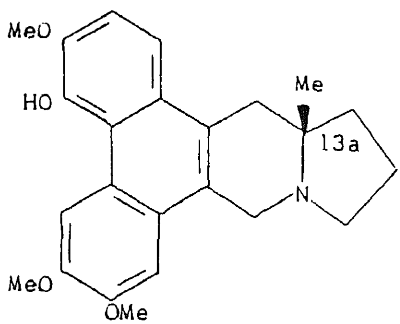
(–)-*Tylophorinicine (14-hydroxytylophorine* (*20*).- A minor alkaloid from the roots of *T. asthmatica*, m.p. 210- 212° dec.; $[\alpha]_D^{25}$ -9.4° (c, 0.04 in $CHCl_3$)[47].

Alkaloids of *T. hirsuta*

Bhutani, Ali and Atal have extensively investigated the aerial part of *T. hirsuta* from Jammu region for alkaloidal constituents[48,49]. In 1984, they have reported the isolation and characterization of five phenanthroindolizidine alkaloids, namely tylohirsutinine (*21*) m.p. 200-202°; 13α-methyltylohirsutine (*23*) m.p. 196- 198°; 13α-methyltylohirsutinidine (*24*): m.p. 213-214°; $[\alpha]_D$ + 120° (c, 0.6 in $CHCl_3$); tylohirsutinidine (*22*), m.p. 234-237°; 13α- hydroxysepticine (*25*), m.p. 290-292° dec. The base *25* on treatment with BF_3-ethearate yielded the dehydrated product *26*. In addition to these alkaloids two bases were also isolated[48]. Ali and Bhutani[49] later on investigated the aerial parts of *T. hirsuta* that grows wild in Jammu region and found to their surprise that none of the five alkaloids namely tylohirsutinine, 13a-methylhirsutinine, 13a-methylhirsutinidine, tylohirsutidine and 13a-hydroxysepticine were present in the wild variety. However, they succeeded in isolation and characterisation of seven minor alkaloids, namely, 14-hydroxyisotylocrebrine (*27*), (+)-isotylocrebrine (*28*), (-)-tylophorine (*29*), 4-desmethyl- isotylocrebrine (*30*), 14-desoxy-13a-methyltylohirsutinidine (*32*), m.p. 180-182°, 5-hydroxy-O-methyltylo-phorinidine (*31*) and tylohirsuticine (*33*). Of these seven alkaloids, the bases *27, 28, 29* and *30* were known and *31, 32* and *33* are new

alkaloids. The struture of the new bases has been assigned with the help of spectroscopic and chemical data[49].

Investigation of the aerial part of *T. hirsuta* cultivated at the campus of the Regional Research Laboratory, Jammu Tawi has also yielded alihirsutine A, a new phenanthroquinolidine alkaloid[50], which has been assigned the structure *34*.



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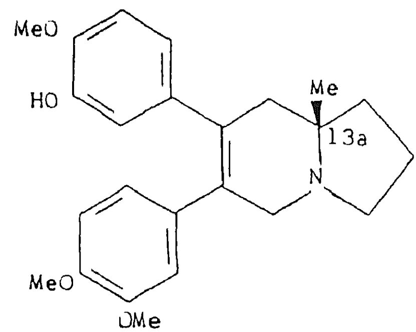

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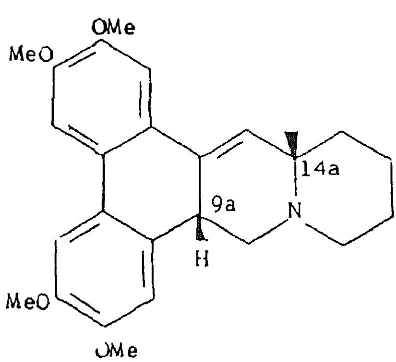

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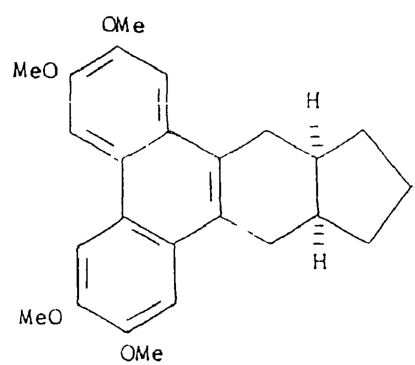

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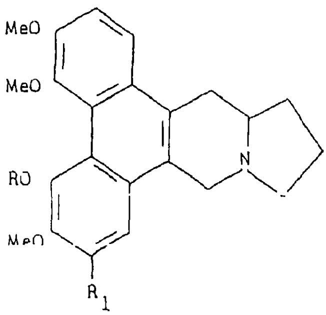
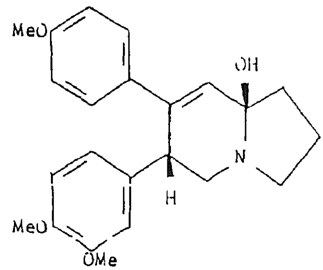


38, R = Me ; R = H
37, R = H ; R_1 = OMe



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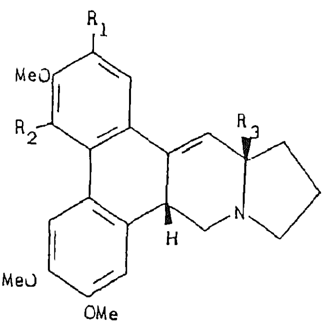
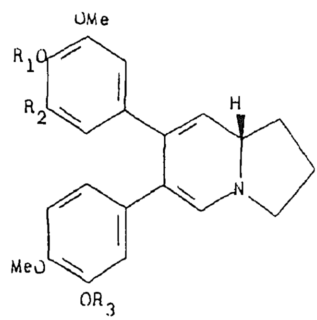


39, R_1 = OMe ; R_2 = H ; R_3 = OH
40, R_1 = R_3 = H ; R_2 = OH



41, R_1 = R_3 = Me ; R_2 = OH
42, R_1 = R_2 = H ; R_3 = Ac

Alkaloids of *T. indica* (Burm. f.) Merr.

T. indica (syn. *T. asthmatica* is a perennial branching climber which grows wild in the plain forests in eastern and southern India. Ali[51] *et al.*, have investigated chemically the aerial parts of *T. indica* collected from the Western Himalayan region and isolated 10 new dihydrophenanthroindolizidine alkaloids, a substituted phenanthrene, named, tyloindane and tylophorine a known phenanthroindolizine alkaloid. The structure of the new bases has been assigned by spectral analysis and chemical reactions[51].

The compounds isolated from *T. indica* are *Tyloindane* (*35*), pale yellow, m.p. 278-279° dec. (MeOH/$CHCl_3$); $[\alpha]_D^{30}$ -0.5° (c, 0.2 in AcOH). *Tyloindicine A* (*36*), m.p. 199-201°; $[\alpha]_D^{35}$ -7.7° (c, 2.1 in MeOH). *Tyloindicine D* (*37*), m.p. 229-231° dec.; $[\alpha]_D^{35}$ + 1.6° (c, 0.6 in MeOH). *Tyloindicine F* (*38*), m.p. 245-247° dec. (Me_2CO/MeOH); $[\alpha]_D^{30}$ -0.5° (c, 0.05 in AcOH). *Tyloindicine G* (*39*), m.p. 237-238° dec. ; $[\alpha]_D^{30}$ -2.35° (c. 0.47 in AcOH). *Tyloindicine H* (*40*), m.p.

225-226° dec., $[\alpha]_D^{30}$ -5.5° (c, 0.55 in AcOH). *Tyloin dine I (41)*, m.p. 215-217° dec. ; $[\alpha]^{30}_D$ -2.3° (c, 0.44 in AcOH). *Tyloindicine J* (*42*), m.p. 180-181° dec. ; $[\alpha]^{30}_D$ -2.85° (c, 0.57 in AcOH).

Biological Activity

At Central Drug Research Institute, Lucknow, the extract of *T. iphisia* Decne collected in June from Nilgiri, Tamil Nadu was put to a broad biological screen. The alcoholic extract was tested for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*; and for antifungal activity against *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes, Aspergillus niger* and *A. fumigatus*. The effects of the extract on respiration, cardiovascular system and on preganglionically stimulated nictitating membrane, isolated ileum and rat uterus, central nervous system and gross behaviour were examined. The extract was also evaluated for diuretic and anti-inflammatory activities. However, the extract did not show any of these activities except that it exhibited good diuretic and CNS stimulant activities. LD_{50} of the extract was found less than 1000 mg/kg i.p. in mice.

T. asthmatica has been used in India as an emetic[52]. The extract of the plant has shown good smooth-muscle relaxant activity *in vitro*[53]. *In vivo* testing established antihistamine and hypotensive activities[53] and also antitumour activity in the L 1210 and P 388 lymphocytic leukemia test system[54]. Shivpuri *et al.*[55], have found that the leaves of *T. asthmatica* are effective in the treatment of allergic diseases, particularly bronchial asthma. Clinical efficacy of asthmatic activity of the material has been confirmed[56].

The anticancer activity of *Tylophora* alkaloids has been reviewed[37]. Gellert and Rudzats[57] in 1963 reported that tylocrebrine (*7*) showed approximately 50% increase in life span (ILS) against the L 1210 leukemia in mice. Test against sarcoma 180 and adenocarcinoma 755 were negative. Data from NCI files show tylocrebrine also to be inactive in the B 16 melanoma, Lewis lung, P 1534 leukemia, and Walker 256 carcinosarcoma tumour systems and to have modest activity in the P 388 leukemia system with increase in life span of approximately 50%[37].

Tylophorine (*4*) was marginally active against the L 1210 leukemia with increase in life span of about 30% and was inactive against the adenocarcinoma 755, Pl 534 leukemia, and Walker 256 tumor systems

Tylophorinine (*5*) was tested only against the L 1210 leukemia and was found marginally active with 20-30% ILS.

Tylocrebrine (*7*) had better activity against the L 1210 leukemia than tylophorine (*4*), tylophorinine (*5*) and hence it was selected in early 1960s for clinical trials. Phase-I trials with 7 were initiated in 1965 but were terminated in 1966 because of

central nervous system toxicity, primarily ataxia, and disorientation. Only few patients were treated to establish the efficacy of the drug[58]

The marginal antitumor activity in active systems, narrow spectrum of activity, and toxicity encountered in the brief clinical study conducted have discouraged further consideration of *Tylophora* alkaloids for development of anticancer drugs

Mode of action.-Tylophorine (*4*) and tylocrebrine (*7*) inhibited protein synthesis in Ehrlich ascites cell but had no effect on nucleic acid[59]. Both the alkaloids *4* and *7* were effective in inhibiting protein synthesis on yeast cytoplasmic ribosomes but no effect on *Escherichia coli* ribosomes even at high concentration. Tylocrebrine (*7*) was shown to have activity equivalent to that of emetine in inhibition of protein synthesis in *Entamoeba histolytica*[60]. The major effect of tylocrebrine is on HeLa cells in inhibition of protein synthesis at the chain elongation stage[61].

Tylophorine and tylocrebrine both inhibit protein synthesis in eukaryotic cells. The anticancer activities of tylophorine, tylocrebrine, and cryptopleurine are less than those of emetine, cephaeline or tubulosine[58]. The mechanism of action of all of these alkaloids is very similar. It is suggested that further development as antitumor compounds should be confined to the emetine series[37].

Tylophorine is lethal to frogs at a dose of 0.4 mg/kg but toxicity to mice and guinea pigs is low. It has a paralysing action on the heart muscles but a stimulating action on the muscles of the blood vessel[52].

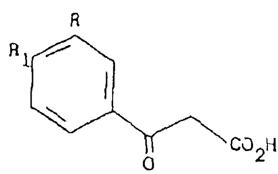
The toxic and vesicant properties of *Tylophora* alkaloids have been noted by several investigators[6,20,62].

Biosynthesis of alkaloids

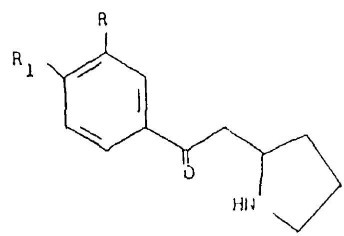
Tylophora species synthesize mainly two types of alkaloids, the seco-phenanthroindolizidine and phenanthroindolizidine alkaloids. (-)-Septicine (*8*) isolated from *T. crebriflora* S.T. Blake, an Australian vine, and *T. asthmatica* Wight et Aru (Syn. *T. indica* (Burm Merril) is a typical representative of seco- phenanthroindolizidine group of alkaloids. Tylophorine (*4*) isolated from several *Tylophora* species is atypical representative of phenanthroindolizidine group. Biosynthetically both the groups are related and have the same biosynthetic origin.

Mulchandani[63] *et al.*, were the first to initiate biosynthetic studies on *Tylophora* alkaloids. Initially it was demonstrated that tyrosine gives rise to ring B and carbon atoms C_9 and C_7 in tylophorine (*4*). In subsequent experiments, phenylalanine, ornithine, benzoic acid and sodium acetate were used. It was found that first two were incorporated efficiently into tylophorine (*4*), whereas benzoic acid did not[64]. Further, sodium acetate was found to be a poor precursor of tylophorine (*4*). Degradation of biosynthetic tylophorine derived from labelled phenylalanine revealed that ring A and carbon atoms C_{10} and C_6, in tylophorine (*4*) were derived

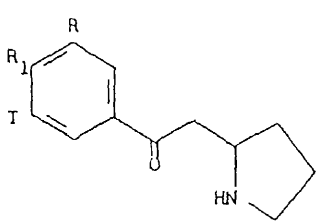
from the amino acid. The efficient incorporation of ornithine suggested its participation in the biosynthesis of *4* probably *via* Δ^1 -pyrroline. Since no degradations were carried out to establish the position of label in biosynthetic tylophorine derived from ornithine, its possible role as a precursor of pyrrolidine ring in tylophorine *(1)* was based on analogy of results obtained in other plants[65]. The results thus established that ring A and carbon atoms C_{10} and C_6, of tylophorine (*4*) are derived from phenylalanine, ring B and carbon atoms C_9 and C_7, of the base (*4*) are derived from tyrosine. Further it ruled out the possibility of shikimic acid- acetate pathway for the biosynthesis of tylophorine (*4*). The poor incorporation of acetate in *4* can result through 'glyoxalate cycle' in which phospho-enol pyruvate, a known precursor of phenylalanine and tyrosine, is formed from acetate.



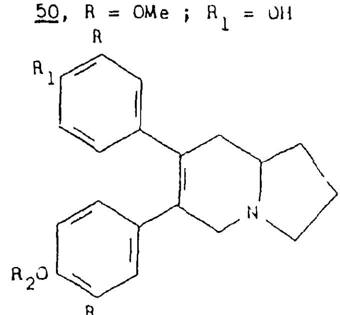
43, R = R_1 = H
44, R = H ; R_1 = OH
45, R = OMe ; R_1 = OH



46, R = R_1 = H
49, R = R_1 = OMe
50, R = OMe ; R_1 = OH



47, R = T ; R_1 = OH
48, R = OMe ; R_1 = OH



51, R = R_1 = R_2 = R_3 = H
52, R = OMe ; R_2 = Me ; R_1 = R
53, R = OMe ; R_1 = R_2 = R_3 = H

Feeding with labelled cinnamic acid in *T. asthmatica* demonstrated that ring A and carbon atoms C_{10} and C_6' are derived from the acid[66]. Herbert *et al.*[67], fed labelled benzoylacetic acid (*43*), 4-hydroxybenzoylacetic acid (*44*), 4-hydroxy-3-methoxybenzoylacetic acid (*45*), phenacylpyrrolidine (*46*), 4-hydroxyphenacylpyrrolidine (*47*), and 4-hydroxy-3-methoxy-phenacylpyrrolidine (*48*) to *T. asthmatica* plants and found that benzoylacetic acid (*43*) and phenacylpyrrolidine (*46*) were incorporated into tylophorinine (*5*).

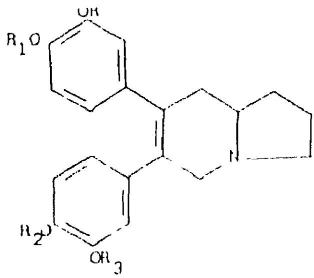
Tracer experiments of Mulchandani, Herbert and their co-workers thus demonstrated that phenylalanine was incorporated into tylophorine (*4*) and tylophorinine (*5*) probably *via* cinnamic acid, benzoylacetic acid (*43*) and phenacylpyrrolidines (*46*), (*47*), and (*48*). The experiments further suggested that hydroxylation and O-methylation in the aromatic ring of phenacylpyrrolidine (*46*) occurred prior to the formation of ring B. Low incorporation of *44* into *5* suggested that hydroxylation in the aromatic nucleus could occur before the formation of *46*.

Herbert *et al.*[67], predicted the occurrence of phenacylpyrrolidine derivatives in the plants on the basis of feeding experiments. The isolation of 2-phenacylpyrrolidine, ruspolinone (*49*) and nor- ruspolinone (*50*) from *Ruspolia hypercrateriformis*[68] proved the correctness of the prediction.

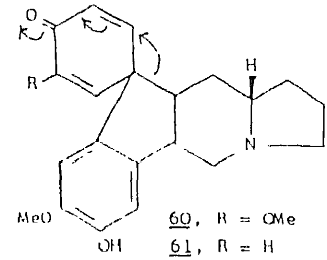
The role of substituted 6,7-diphenyl-1,2,3,5,8,8a- hexahydroindolizines in the biosynthesis of tylophorine (*4*) and tylophorinine (*5*) was then studied by Herbert and Jackson[69] by feeding of substituted phenylhexahydroindolizines *51*, *52* and *53* to *T. asthmatica* plants. It was demonstrated that indolizine derivatives *52* and *53* were incorporated into tylophorine (*4*) and tylophorinine (*5*).

Bhakuni and Mangla have studied in detail the late stages of biosynthesis of tylophorine (*64*) and tylophorinine (*67*) in *T. asthmatica* plants. Administration of 3,4-dihydroxyphenyl [2- ^{14}C] alanine to young *T. asthmatica* plants revealed that carbon atom C_9 and C_7 of tybophorine (*64*) and tylophorinine (*67*) are derived from dopa. Tracer experiments with 6,7-diphenylhexahydroindolizine (*54* - *59*), demonstrated that compound *54* is efficiently and specifically incorporated into tylophorine (*64*) and tylophorinine (*67*). Compounds (*56*) and (*58*) were not metabolized by the plants to form *64* and *67* whereas *58* and *58a* were utilized to yield (*64*) and (*67*). Compound (*55*) was very poorly converted into (*64*) and (*67*) and thus is not on the major biosynthetic pathways of (*64*) and (*67*). The experiments thus strongly suggest the biosynthetic pathways of tylophorine (*64*) and tylophorinine (*67*) in *T. asthmatica* as follows: Phenylalanine + Dopa ⟶ (*58*) ⟶ (*58*a) ⟶ (*54*) ⟶ tylophorine (*64*) and tylophorinine (*67*).

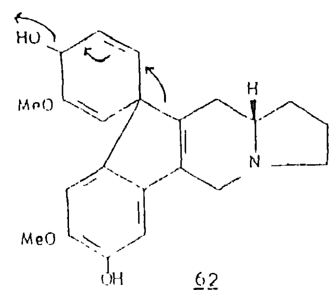
The biosynthesis of septicine (*8*) and related alkaloids, although not yet studied, must surely be intimately related to the biosynthesis of the phenanthroindolizidine alkaloids *54*. The key intermediate of these alkaloids should furnish septicine (*8*) by O-methylation. Study of biosynthesis of tylophorine (64) using callus tissues from *Cynanchum vincetoxicum* is under investigation[70].



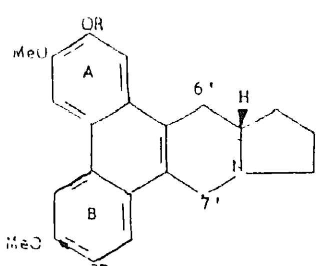
54, $R_1 = R_3 = H$; $R = R_2 = Me$
55, $R = R_3 = H$; $R_1 = R_2 = Me$
56, $R = R_2 = H$; $R_1 = R_3 = Me$
57, $R = R_1 = R_2 = R_3 = Me$
58, $R = R_1 = R_2 = R_3 = H$
58a, $R = Me$; $R_1 = R_2 = R_3 = H$
59, $R_2 = Me$; $R = R_1 = R_3 = H$



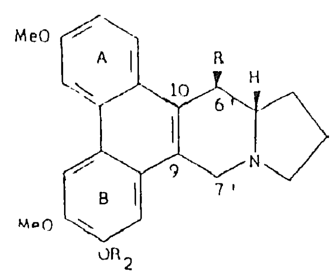
60, R = OMe
61, R = H



62



63, R = H
64, R = Me



65, $R = R_2 = H$; $R_1 = Me$
66, $R = H$; $R_1 = R_2 = Me$
67, $R = OH$; $R_1 = R_2 = Me$

Fig 1. Biosynthetic pathways of tylophorine (64) and tylophorinine (67)

Tissue Culture

Initial attempts to regenerate cell line of *T. indica* by tissue culture to obtain alkaloids of interest did not succeed[71]. Subsequently, the alkaloidal profile and concentration of the alkaloids in tissue culture regenerated plants were found similar to those vegetatively propagated field grown plants of *T. indica*[72].

Effects of Nutrients on *T. indica*

Effect of micronutrients on growth and alkaloidal contents of *T. indica* has been studied[73]. Further studies have been carried out on nutritional requirements of the crop for higher yield of alkaloids. It has been observed that the application of nitrogen influenced herb as well as total alkaloid content of the plant. Significant increase in herb yield was obtained at 150 kg/ha. However, 150 kg nitrogen in combination with 50 kg P/ha gave maximum increase in herb/plant as well as total herbage/ha after two harvests. Higher concentration of nitrogen i.e. 150 kg and 200 kg/ha alone and also in combination with phosphorus at 50 and 100 kg/ha were effective in increasing total alkaloid content of the plant. Phosphorus alone did not show any effect on either of the yield parameters[74].

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*Cultivation of *Ammi majus* Linn. in India

B.L. Bradu

Regional Research Laboratory, Jammu Tawi

Introduction

AMMI MAJUS Linn. (Apiaceae) is an important medicinal herb, occurring worldwide in both the hemispheres. The plant is indigenous to Egypt and widely distributed in Mediterranean region, Abyssinia, Europe and West Africa (Blatter, 1923). It constitutes principal commercial source of xanthotoxin, which is commonly used in the treatment of vitilago or leucoderma and also in formulating sun-tan lotions. In India, the plant was first introduced from Egypt at Forest Research Institute, Dehradun in 1955. Since then its experimental cultivation has been tried in several parts of the country. Its experimental cultivation in Chakrata Forest division, Dehradun gave an average fruit yield of 375 kg/hectare, and its low yield was suggested to be due to excessive rainfall (Singh, 1963). According to Singh (*loc. cit*) France, Switzerland and Federal Republic of Germany required about 400 tonnes of *Ammi majus* fruit annually, part of the requirement met from Indian export. There has been steady increase in the cultivation of *Ammi majus* crop in India and as a result the country meets the demand for internal consumption of the drug and export the finished product as well. Mac Laboratories, Bombay and Himalaya Drugs Ltd., Delhi were pioneers in the drug production and mainly met the Country's export demand.

In view of the low yield reported in Dehradun, Bradu and Atal (1970) undertook its first experimental cultivation in Jammu and later at Palampur and

Baijnath (H.P.) registering its success and proved the economic viability of the crop. This finding led to its commercial growing in various parts of north India.

The exotic species was biennial, but behaved as annual under cultivation in India. The plants, also called as Greater Ammi or Bishop's weed, is an erect branching herb, upto 1.5-2m in height, leaves broad ternate or pinnate, leaflets lanceolate, flowers white in loose compound umbels, fruit oblong, 1.5-2.0mm long and 1mm or less broad. Under large scale cultivation in various parts of India, the growth remained vigorous, prolific in flowering and fruiting. When cultivated at CIMAP drug farm, Chakrohi (Jammu), Duhan *et al.*, (1986) reported that crop should be grown in the second fortnight of October with 90 kg N/ha for obtaining good quality seed yield. Gupta and Shah (1988) also reported October sowing of the crop with higher xanthotoxin content. Randhawa *et al.*, (1986) found that fruit yield was not affected statistically with increase in nitrogen level from 50 to 100 kg N/ha. Adam *et al.* and Shehab *et al.*, (1985) reported respectively, the cytological effects of water extracts of *Ammi majus* on the mitosis and meiosis of *Vicia faba*. The extract caused a delay in the completion of the mitotic cycle. While the interphase stages remained uneffected, but prophase and metaphase stages were most influenced. The dominant abnormalities were stickiness, irregular prophase, bridges with low frequency lagging prophase metaphase and despiralization. In meiosis the anomalies in the first division were higher than in second. The induced irregularities were stickiness, bridges, spindle disturbance, lagging chromosome, breaks, fragments and multinucleate cell. The plant extract caused a decrease in pollen grain length and slightly increased pollen viability.

Greater Ammi contained 0.4% xanthotoxin and additional quantities of xanthotoxin can be prepared by converting imperatorin present in the fruit to xanthotoxin by chemical means (Ganapathi *et al.*, 1970). Wealth of India, Raw Materials, Vol.I: A (revised), (1985) quoted that xanthotoxin was effective in the treatment of leucoderma when given orally in doses of 50 mg, three times daily or applied externally as one percent liniment followed by exposure of affected areas to sunlight or ultravoilet light for 2-4 hours. It further added that xanthotoxin (ammoidin) was the Chief active principle in the fruit. It was a furocoumarin marked under the trade mark 'oxsoralen'. Other furocoumarins present were ammidin (imperatorin), majudin (bergapten), marmesin, isopimpinellin, majurin, ammajin (marmesinin), isoimperatorin, ammirin, and 8-isopropenyl-8,9-dihydroangelicin (probably angenomalin). The presence of a non-furocoumarin umbelli-prenin (mp 60-61°), glycosides of quercetin and luteolin and two artefacts 7-isopropyl-psoralene and dihydro-oroselone. Fruits contained protein (13.8%) and fat (12.9%). The presence of xanthosine (mp 144.47°) was reported in the seed. The leaves contained xanthotoxin and isopimpinellin. A method claimed to be economical was developed at Regional Centre of CIMAP at Jammu. In this method, the seed was cleaned, powdered and extracted with solvent oil (80-100°). The coumarins recovered from the extract by chilling and liquid-liquid extraction, was subjected to chroma-

tographic separation after treatment with alcoholic hydrochloric acid. Bergapten, xanthotoxin and xanthotoxol were separated and the xanthotoxol fraction methylated, and the total xanthotoxin (also known as methoxsalen) obtained was purified by charcoal treatment in acetone or alcohol. Ashraf *et al.*, (1979) examined the seed essential oil of Pakistani *Ammi majus* for its quality and chemical composition. He reported the percentage essential oil constituents which comprised high boiling hydrocarbons (1.34%), dl-piperitone (10%) unsaturated cyclic terpene alcohol (15%) and a mixture of furocoumarins (60%). He found that furocoumarins fraction was composed of xanthotoxin, bergaptene, imperatorin, iso-imperatorin and iso-pimpinellin of which xanthotoxin was the major component. Wayne (1978) reported twelve linear furocoumarrins (psoralens) isolated from the ripened seed of Texas (U.S.A) collection of the photosensitizing Bishop's weed. Four of the compounds i.e. xanthotoxin, bergapten, isopimpinellin and iso-imperatorin had already been isolated, but isolation of six of the eight other psoralens, which were known plant products, but not obtained earlier from *A. majus*. The rest of the two psoralens i.e. 8-(2- (3-methyl-butyroxy)-3-hydroxy-3-methyl butoxy) psoralen, and 5-(2- (acetoxy) -3-methyl butoxy) psoralen, not reported earlier from any plant source, were isolated from *A. majus*. The author further reported that most of the major psoralens from *A. majus* were potent photosensitizers, based on bioassays against chick skin and the presence of these compounds in seed and other parts of the species accounts for high photosensitizing activity towards cattle and sheep, besides reporting their use in the treatment of psoriasis. Witazel *et al.*, (1978) also reported occurrence of *A. majus* on the coastal region of Southern USA and in other parts of world, causing severe photosensitization in livestock and contributing to its outbreaks in Texas (U.S.A). Experimental trials conducted by him on sheep fed with finely ground seed of *A. majus* at dose rates of 1-8g/kg of body weight and exposed in sunlight. A single dose of 8g/kg produced severe clinical signs in 24-48 hours, resulting in corneal edema, conjunctivo-keratitis, photophobia and edema of the muzzle, ears and vulva. Such severe long term photosensitization effects were reported by various other authors. Mendez *et al.*, (1991) also reported an outbreak of primary photosensitization in cattle grazing on pasture in Southern Brazil invaded by *A. majus* weed, showing clinical signs characterised by dermatitis in the Udder of the cows and kerato conjunctivitis in the calves.

In the heterotrophic cell suspension initiated from hypocotyl of young *Ammi majus* seedlings, Hamerski *et al.*, (1990) reported *A. majus* cells suitable for biosynthetic studies on various coumarins as well as for regulatory studies of the inhibition of coumarins phytoalexin accumulation.

Cultural Practices

Ammi majus could be grown in a variety of soils but does well in a loamy soil. The soil is ploughed twice and brought to a fine tilth. The seed being very fine is mixed with fine soil or sand before sowing in shallow furrows 90 cm apart. After sowing,

the seed should be covered with a fine layer of soil. The seed rate is 2.5 kg per hectare. The seed can be sown from Ist of October to middle of November, but the seed sown at the end of November generally fail to germinate. A dose of super-phosphate at 25 kg/ha mixed with liberal quantity of farm yard manure is applied in the furrows before sowing the seed. If it does not rain within a week after sowing, a liberal irrigation is given. The seed starts germinating after a fortnight and takes about a month to complete the germination. When the seedlings develop true leaves and are 8-10 cm in height, thinning is done so that plant to plant distance within the row is 60-90cm. Two hoeings are given to keep the soil mellow and free from weeds. Irrigation is given after a week or ten days during the dry season. Water should not be allowed to stand in the field as the plants are sensitive to water logging.

Harvesting

Bradu and Atal (1970) studied the best stage for harvesting the fruit when xanthotoxin was maximum. Mature brown fruit contained 0.4%, mature but green fruit 0.72% and immature green fruit 1% of xanthotoxin. This suggested the best stage of harvesting the fruit. The highest yield of xanthotoxin, imperatorin and bergapten from the air-dried mature unripe fruits from Cairo (Egypt) was reported to be 1.06, 0.8 and 0.33% respectively. Bradu and Atal (1970) found that delay in harvesting caused considerable loss in fruit yield due to shattering. Sobti *et al.*, (1978) studied the problem of shattering of fruit in *A. majus*. Planofix (active ingredient NAA) at concentration of 5 ppm was applied at 2 stages, one at the initiation of flowering and second at the time of formation of fruit, in an area of 0.48 hectare. The fruit yield of 1500 kg/ha was obtained as against 900-1000 kg/ha under control, an increase of 50% over control. Uniformity in flowering and maturity of umbels was obtained to a considerable extent, resulting in easy harvesting.

The flowering starts in the beginning of March and fruiting by end of April. The primary umbels fruit first and are hand picked towards end of April or early May when majority of remaining umbels (secondary and tertiary) are about to mature (at mature green stage), the crop is harvested, cut at middle level, and stacked in loose bundles till fruits dry up. The fruit is separated by thrashing and winnowing. A yield of over 1375 kg/ha was obtained under experimental conditions and 900-1000 kg/ha in large scale cultivation under Jammu conditions. In Palampur-Baijnath area, the fruit yield was 600 kg/ha, but the low yield was compensated by higher xanthotoxin content (1% and above).

Rotation

A cultural trial of *Heracleum candicans* was also tried at Palampur. The seed was sown in nursery beds in March and seedings were transplanted towards end of April or beginning of May in those fields where *A. majus* was grown. The growth became

vigorous during rainy season and crop started flowering in July. The crop was harvested after 21 months. Being a root crop, the roots were extracted out in December following year. The fresh weight of root on an average was 1.2 kg per plant having a total coumarin content of 8.5%.

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*Heracleum candicans Wall. – A Potential Source of Xanthotoxin

M.K. Kaul

Regional Research Laboratory, Jammu/Srinagar

Introduction

XANTHOTOXIN is an important drug extensively used in the preparation of sun-tan lotions and to some extent for the treatment of leucoderma. It is in demand throughout the affluent countries. The principal source of this drug has been *Ammi majus*, an Egyptian herb, with a total yield of about 0.41 per cent Xanthotoxin (Bradu & Atal, 1973). The genus *Heracleum* was reported to contain furanocoumarins which are convertable to Xanthotoxin (Handa and Rao, 1970)[2]. This plant is distributed mainly in north temperate region and some tropical mountains (Hooker, 1879)[3]. According to an estimate every year 150 tonnes of fresh roots of *H. candicans* are extracted from N.W. Himalayan region. During 1980-1985 about 700 tonnes fresh roots have been extracted from wild sources in Kashmir Himalaya (Kaul, *et al.*, 1985)[4]. The ruthless extraction of this herb, particularly *H. candicans*, threatens its extinction in years to come. This paper details our studies on domestication and different aspects of agrotechnology for improvement of xanthotoxin content in this herb. Data are presented on distribution pattern, seed germination, germplasm evaluation, total coumarin content at different stages of growth, manurial requirements, yield parameters and better methods of clonal propagation of high xanthotoxin yielding plants.

* Revised and updated.

Germination Studies

(a) Chilling effects on seed: The seeds do not germinate as such. An experiment was laid to study the effect of chilling on the seed germination. Freshly collected mature seeds were chilled under different temperatures in the laboratory. These were wrapped in moist filter papers and three ranges of temperatures were selected i.e. below freezing (-2° to 4°C), freezing (0°C) and above freezing (2° to 5°C). The seeds were exposed to these temperatures for periods varying 15 to 75 days. Later the germination was studied in temperature ranges of 10°, 15° and 20°C.

It was found that seeds need six to eight weeks of chilling at 2° to 5°C before the commencement of germination. The optimum germination after chilling was achieved at 15°C.

(b) Extent of seed dormancy: Mature seeds of five age groups (1-5 years age) were used for chilling experiments. The germination of these seeds was studied in temperature range of 10°-20°C. Standard practices of germination were followed.

It was noticed that there is a considerable reduction in the germination capacity of seeds with the advancement of age. The seeds are found to retain dormancy for a period of three years if properly stored.

Vegetative Propagation

Rootlets arising from the main rootmass were washed and cut into 2-3 pieces. Lower part of each piece was dipped in Seridex 'B' root hormone and planted in moist soil. Rooting was initiated and new plants were obtained. This method was adopted for clonal multiplication of high Xanthotoxin yielding strains.

Agrotechnological Studies

Standard agronomic experiments were laid to study the irrigation, spacing, fertilizer and manurial requirement of this new crop. The increase in rootmass over a period of three years was studied in the experimental plots of Srinagar laboratory. Sampling of roots from the plant population was made at different stages of growth. These samples were analysed for total coumarins to ascertain the right stage of harvest in this crop. There is not much difference in the content of total coumarins at pre- and post-flowering stages of plant growth in *H. candicans* (Bhat & Kaul, 1979)[5].

An experiment of spacing revealed that seedlings should be planted at a distance of 75cm. × 50cm. to obtain the optimum yield (Kaul, 1983)[6]. Fertilizer trial revealed that application of 25 tonnes of farm yard manure per hectare gives the optimum yield of roots (Bhat & Kaul, 1979).

An average yield of 300 kg (dry weight root) per acre was obtained in the first year of growth and it was seen to increase to 425 kg, if the roots are harvested after a period of two years. No appreciable increase in root growth was observed in third year (Bhat & Kaul, 1979).

In cultivation plants develop a number of rootlets as compared to the wild plants. These rootlets were found to be rich in total coumarins than the main rootmass. On an average the total coumarin content is double in rootlets as compared to the main rootmass. (Kaul *et al.*, 1982)[7]. The root cuttings were successfully propagated. The optimum yield of xanthotoxin in wild stock has been found to be 1%. Under cultivation there is an improvement in the Xanthotoxin content of mature roots. The otpimum yield in cultivated stock on an average was found to go up to 1.5%, although high xanthotoxin yield (upto 2.5%) was reported in certain plants which were selected and propagated vegetatively (Kaul, 1989)[8].

Germplasm Evaluation

In view of the fact that this herb possesses lot of variability in nature, a wide germplasm was collected from different regions in North-West Himalaya and grown under similar conditions at Srinagar laboratory. All the phenological data like height of plant, leaf structure, flower and fruit structure, flowering period, and seed output was recorded for statistical analysis of variability. Based on the flowering pattern and leaf structure two races were identified:

(a) Early flowering race: In these plants flowering occurs in May. It has two types: broad-leaved type and narrow leaved type. Both types have a thoroughly branched root system. The broad-leaved type is a heavy seed-setters as compared to narrow-leaved type. On an average total coumarin content of this race is 9%. The narrow-leaved type, although not robust, gives better yield of coumarins.

(b) Late flowering race: These plants flower in July. It does not possess much branched roots and produces less seed. On an average total coumarin content is 6% (Kaul, 1989)[8].

Conclusion

From these studies the following conclusions are drawn:

1. *Heracleum candicans*, is a potential indigenous source of xanthotoxin.
2. It is superior to *Ammi majus*, an Egyptian herb, so far as the yield of Xanthotoxin is concerned.
3. *H. candicans* can be brought under cultivation without much difficulty. This will greatly ensure a constant supply of quality raw material to the user industry.

4. Lot of variability exists in the total coumarins content of different plants from different locations. The variability can be exploited usefully.
5. Clonal propagation of superior genotypes has been made possible by the use of root hormones.

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*Cultivation of Rutin Bearing Eucalyptus Species

R.P. Sood, N.K. Kalia**

CSIR Complex Palampur - 176 061 (H.P.)
**RRL Research Station, Palampur - 176 061 (H.P.)

RUTIN, 3,5,7,3′, 4′ pentahydroxy flavone-3-rhamnoglucose is known as 'P' factor or a substance possessing vitamin P like activity. Rutin was first isolated from *Ruta graveolens* (1).

Rutin is an important pharmaceutical product. It exerts a wide range of pharmacological effects (2). It is reported to be useful in the treatment of capillary fragility, and possibly rheumatic fever of haemorrhagic conditions. Generally, capillary fragility occurs in a significant number of cases of high blood pressure, diabetes and other conditions. It is reported to be effective in restoring to normalcy such capillaries in many cases. It is also useful as aid in the prevention of apoplexy and retinal haemorrhage in those cases where capillary fragility is concerned. It is used in the case of coronary thrombosis and purification of blood. Rutin has also been shown to provide protection against harmful effects of X-rays, indicating that it may be of use to the persons exposed to dangerous atomic radiations. (3). The effect of rutin on the cardiovascular system was studied and it was reported that the compound increased the stroke volume of both intact and isolated frog's heart (4). Under certain conditions, rutin can afford animals protection against histamine shock (5). Rutin has been shown to be of value in protecting rabbits from frost bite (6). Rutin can also exhibit the properties of hyaluronidase, especially with ascorbic acid, like hesperidin which incidentally works as an oral contraceptive (7). It is

*Revised and updated

found that only rutin prevented sexual reproduction, which they attributed to the existence of a sugar molecule at position 3, because quercetin did not influence fertility. But this fact has not been substantiated by other workers (8). Rutin can also be used as an analytical reagent and quercetin can be prepared from it in a high state of purity by simple acid hydrolysis. Rutin has been used clinically in treating certain types of hereditary hemorrhagic disorders (9), hemophilia symptoms, bleeding gums, migrainic headache, oedema, hydro-cele, hematuria, toxaemia in pregnancy (10) (11). The fat anti-oxidant properties of these flavonoids have also been described (12). Hence, its hydrolysed product can be very useful for preventing oxidation in milk, butterfat, ghee etc (13). Recently, some preparations incorporating Rutin have claimed improvement in patients testing +ve for HIV and showing the symptoms of AIDS and AIDS related syndromes (14).

A number of investigators have since found rutin, usually in small quantities, in a considerable number of plant species. A list of only those plants having more than 5 percent rutin is given in Table - 1 (15,16,17).

The important pharmaceutical product is not produced indigenously in sufficient quantity and is imported to meet the internal requirements of the industry. The

Table 1 — Plants containing more than 5 per cent rutin

Family	Species	Rutin (%)	Parts of plant
Myrtaceae	*Eucalyptus macrorhyncha*	10.00-24.0	Leaves
	E. youmanii	6 8-11.0	Leaves
	E. delegatennsis	4.0-6.0	Leaves
Leguminosae	*Acacia dealbata*	5.2-5 9	Flowers
	Sophora japonica	13.0-30.0	Flowers
Caprifoliaceae	*Sambucus canadensis*	5 2	Immature Flowers
Papaveraceae	*Escholtzia californica*	5.0	Flowers
	Hypecoum pendulum	5.0	Flowers
Polygonaceae	*Fagopyrum cymosum*	4.0-8.5	Leaves and
	F. esculentum	0.11-6.37	Flowers
Rutaceae	*Boronia serrulata*	6.80	Leaves
	B. thujona	5.00	Leaves
Violaceae	*Viola lutea*	16.6	Flowers
	V. tricolor var. maxima	18 3-21.2	Flowers
	V. odorata		
Anacardiaceae	*Mangifera indica*	5.2	Leaves
Rubiaceae	*Uncaria elliptica*	20	Leaves

total annual demand is about twenty tonnes of rutin and the present market rate is around Rs 1200-00 per kg. The total world annual requirement is approximately of the order of 37-40 tonnes about a decade ago which must have risen considerably over the years.[18].

Two important rutin enriched plant species of *Eucalyptus youmanii* and *E. macrorhyncha* have been introduced and domesticated successfully in Kangra valley region of Himachal Pradesh (19). The leaves of these plant species are rich in rutin content. The trial planting of *E. macrorhyncha* was undertaken in the year 1963-64 in the Nilgiri and Palani hills in South India (20). *E. youmanii* has been introduced in H.P. from Australia by this group for the first time. The preliminary trials made at Palampur (H.P.) have indicated that these species are very promising and valuable crops for this region (76.3° longitude and 32.6° latitude, altitude 1350-1400 metres above M.S.L.)

E. youmanii is moderate sized tree, commonly 12-20 metre in height, and diameter of 0.45 to 0.90 metre. It is usually heavily branched form about 1/3 height and has a moderately large to large, dense crown and grown on the low hills of northern tablelands of NSW in Australia in sclerophyllous Eucalyptus forests. The adult leaves are alternate, stalked, tending to narrow lanceolate, oblique, slightly falcate, lateral veins visible on one side, faint on the other, moderately thick, coriaceous, green in colour and concolorous. It has stringy bark; thick on the lower part of the trunk, grey to reddish-brown in colour, and persistent to the smaller branches.

E. macrorhyncha is commonly known as red stringy bark and sometimes mountain stringy bark. It is medium-sized tree maintaining a height of 21-30 metre; 0.45-105 metre diameter, with bark, as its common name implies that it is dark reddish to brown, persistent and stringy. The plant is indigenous to the sclerophyll forest of the central and south western slopes of N.S.W.; Victoria and in South Australia.These regions are subject to hot spells and droughts. It grows there between 300-900 m above MSL in Australia. The leaves of the mature tree are typical of the genus. They have a very thick cuticle and the laminae are borne vertically downwards. The leaves are alternate, petiolate, narrow, lanceolate and somewhat venulose. The leaves contain oil glands and tannin cells. This tree carries a moderately less dense crown than *E. youmanii*. The life span of *E. macrorhyncha* leaves is short, compared with evergreen trees of other genera. Leaf fall from *E. macrorhyncha* tree is a constant process and is greatest at the commencement of periods of fresh growth and when the fruit is forming. The mean life of the leaves is about 18 months. It varies with their position on the tree as on vigorous branches, many leaves fall during the first year and most of the remainder leaves fall when the next burst of growth commences (21).

E. youmanii and *E. macrorhyncha* were raised from seeds by first raising a nursery followed by transplanting the seedlings. Nursery sowing is generally preferred as the tiny plants require intensive care to protect them from frost, severe

cold and winter rains. It takes about six months for the plants to gain a height of 15-25 cm when they are transplanted in the field. Seeds of *E. macrorhyncha* are smaller (720 seeds/g). They normally lose viability on ageing and fresh seeds are used for raising nursery. Germination percentage of *E. macrorhyncha* is almost double than *E.youmanii* under Palampur conditions. The germination percentage of both the species is, however, lesser than that in Australia.

Seeds were obtained from several agencies in Australia in successive years and their performance noted under local conditions. The best time of sowing the seed in Jan-Feb. (Table 3).

Seedlings raised during this period were healthy, less prone to diseases and could attain considerable height before their transplantation during mid-monsoon period . During the hot summer months of April-May, the seed germination is poor and the seedlings which emerge suffer heavy mortality due to damping off, stunted growth and chlorosis. The germination is fairly good during June-August; but plant growth is stunted in pre-nursery stage, during the long severe winter in this region.

Table 2 — Viability of Eucalyptus Seeds

Plant	No. of seeds per 10 gm	Seed Viability			
		Palampur (India)		Australia	
		per seed lot of 10 gm	No. of seed lots tested	per seed lot of 10 gm	No. of seed lots tested
E. macrorhyncha	7200	450	4	600	-
E. youmanii	4250	215	6	240	-

Table 3 — Relative Germination Performance (uncontrolled Conditions)

Period/Month	*E. macrorhyncha* Time of germination	% Germination	*E. youmanii* Time of Germination	% Germination
Sept.-Oct.	24	-	35	-
Nov.-Dec.	30	4.30	36	2.25
January	24	4.85	27	3.25
February	25	5.55	25	3.15
March	10	3.55	29	3.50,
April-May	11	3.00	14	4.00
June-Aug.	7	4.00	10	3.85

Seeds were sown shallow in alkathene bags, packed with well mixed soil and sand enriched with organic matter in Feb. The seeds, being very small in size, when sown deeper than 1 cm did not germinate. General screening of healthy seeds was done before sowing. Corresponding to the viability figures, an average of 10-12 seeds of *E. macrorhyncha* and 15-18 seeds of *E. youmanii* were sown in each alkathene bag to ensure uniform germination. The extra seedlings were picked and transplanted in separate bags. Alkathene bags were covered with a layer of paddy straw just after sowing the seeds to ensure adequate moisture and heat conservation. The straw layer was removed after the commencement of germination.

The seeds were also sown in wooden or aluminium trays by spreading them on the surface and covering with thin layer of fine soil. The tiny plants, having opposite leaves were picked out with care and transplanted in alkathene bags. The trial was repeated by keeping the trays in small polythene covered air tight chambers to ensure uniform and constant humidity and optimum temperature for quick and better germination. This practice was found to be convenient and successful and is recommended for large scale planting.

Seeds of *E. macrorhyncha* germinated within 20-25 days, while those of *E. youmanii* took comparatively more time under uncontrolled conditions.

Regular irrigation of nursery plants in bags, protection from outside cold and frost at night and conservation of moisture was found to be essential. It was done by making bamboo sheds over the nursery beds in the beginning. Regular weeding was also important as the two species are very sensitive to weed during the nursery stage.

In another trial on germination performance, the seeds were treated with varying concentrations of gibberellic acid and kept soaked overnight before sowing. In *E. youmanii*, the period of germination of seeds was reduced considerably whereas germination percentage was increased three times over the control at 50 ppm concentration. Lower concentration of the growth regulator had little effect on the overall performance of *E. macrorhyncha*. Under semi-controlled conditions (Poly chambers) the germination time was reduced to 10-12 days only for the two species.

Evidently, growth was faster in case of *E. macrorhyncha* as compared to *E. youmanii* during the post-nursry stage. The growth of *E. youmanii* was vigorous after transplantation. March to early June is comparatively hot in Palampur and partial protection of plants, still in nursery stage, from the hot weather was made by covering the alkathene bags with bamboo netting. The alkathene sheet was removed during this period. Careful hoeing around the plants to a moderate depth of about 2 cm could help in sufficient weed control. It also helped in improving soil aeration which resulted in better growth of seedlings. But it is desirable to apply this practice when the plants have fully established.

The plant species are very susceptible to termite damage which is increased when the plants lose vigour. To eliminate such attacks, incorporation of prophylactic doses of BHC powder or aldrin dust in the soil during the nursery stage was found necessary. Such applications were, at times, needed after transplanting also. Leaf sucker attacked these species during monsoon and spray of 0.05 percent laby-acid in pre and post-planting stages, controlled the damage.

Pits of $15 \times 15 \times 15$ cm^3 size were made in the field at spacing of 1.5×1.5 m^2 for the transplantation of seedlings. The soil was well mixed with adequate quantity of FYM and NPK as basal dose. The transplanting was done towards the end of monsoon in late July-August. Since water logging increased the mortality rate of young seedlings, interchannels were made in the plots to remove excess of water, during rainy season. After planting, the area around each plant was cleaned thoroughly. Coppicing was done to a moderate plant height of 1.5 metre to make a dense crown. An adequate dose of German mixture (NPK 15:15:15) was incorporated in the soil around each plant during the year in split doses. *E. youmanii* grew very profusely under Palampur conditions. The leaves were more healthy, dark green in colour and denser than those of *E. macrorhyncha*.

Some experiments were carried out on the different applications of inorganic fertilizers, liquid foliar fertilizers and *n*-triacontanol (22). Results indicated that the application of 312 kg N/ha (urea) in two equal split doses resulted in a yield of 446 q/ha of dry leaves. The yield of first harvest was higher as compared to the second harvest. This is attributed to the better growth conditions during the period of February to May in this region. The percent increase in total herb yield over the control was 21.9%. Still higher doses of nitrogen did not produce linear results due to the lack of irrigation.

Various levels of foliar fertilizer when sprayed six times a year resulted in a linear increase of herb yield upto 0.30%. The average dry herb yield per plant with 0.30% treatment was 595.0 g in comparison to 398.0 g/plant under control. Thus on hectare basis, the net leaf biomass increase was 49.6% over the check.

An application of 10 ppm of n-triacontanol four times a year resulted in an increase of 51.3% leaf biomass, over the control. Further with the application of 20 ppm dose, there is not significant improvement in the annual herb yield. Earlier reports on the stimulation of growth by this hormone in several field crops are mentioned (23), (24), (25), (26), (27), (28), (29). The increase in growth (dry weight) after foliar application of *n*-triacontanol is attributed to the factors like increase in chlorophyll concentration and leaf area (30), its effect on some process which regulates the balance between photosynthesis and photorespiration (31) and reduction in photorespiration, delayed senescence and increased level of photoassimilates (30).

Two species are grown in Australia in less fertile lands and are reported to improve the texture and fertility of the land. *E. macrorhyncha* prefers low altitude

between 300-900 m and does not show juvenile growth as compared to *E. youmanii* when planted on higher altitudes. This was noted in the trial plantation undertaken at different heights ranging from 500-1500 m to find out the optimum altitude for large scale cultivation of the two species in the sub Himalayan regions in H.P.

Harvesting

E. youmanii performed very well at high altitude between 1000-1500 m in the upper Dhaula Dhar range of Himalayas whereas the growth of *E. macrorhyncha* was stunted at this range. *E. macrorhyncha* could, however, be grown well below 800 m. height in lower hills to get optimum yields. A record of herbage of the two species from six year old plantation is given in Table 4, below:

Evidently, the yield of *E. youmanii* was less than that of *E. macrorhyncha*. The rutin content in the leaves of two species of three year old plantation was also studied at one month interval.

The maximum rutin content was obtained in the month of July. It was also observed that rutin percentage was more in young leaves near the tip of the twig than in mature leaves Table 6 below:

Extraction of Rutin

Rutin is commercially extracted with hot water at higher temperature. It is fairly soluble in boiling water and starts settling down at about 90°C and adequate care is needed to filter the hot water extract. Fine powdered dry leaves 30-40 mesh are boiled with water (1:20) for about 25-30 minutes in closed vessel at elevated pressure of 15-20 lbs. Excessive heating for long period causes hydrolyses of Rutin

Table 4 — Herb Yield From Six Years Old Plantation

	No. of Plants (Ha)	Dry Leaves (T)
E. macrorhyncha	4400	4.140
E. youmanii	4400	3.900

Table 6 — Distribution of Rutin Content along the Twig

Distance from tip (cm)	2.5	5.0	7.50	10.0	12.50	15.0
Rutin (%) in *E. macrorhyncha*	11.50	7.00	6.50	5.50	3.50	2 50
E. youmanii	13.50	9.00	8.50	6.00	3.50	2.00

(Rutin content on moisture free basis)

Table 5 — Periodic Variation of Rutin Content . 3 Years Old Plantation

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
E. youmanii Rutin (%) (MFB)	10.40	10.20	11.05	10.85	11.80	11.20	15.70	13.60	11.35	13.95	12.10	11.80
Moisture	54.50	54.20	53.00	46.00	49.00	55.00	58.00	58.00	50.50	54.50	58.00	51.70
E. macrorhyncha	1.80	8.12	7.40	8.05	8.04	9.60	11.10	9.30	8.45	9.40	9.40	9.10
Rutin (%) (MFB) Moisture	53.40	54.10	50.00	52.00	51.00	50.00	49.50	44.50	48.00	50.40	51.00	49.00

into Quercetin. The hot extract is filtered immediately and fine yellow coloured rutin is separated out on cooling. The crystalisation of pure golden yellow coloured rutin is done with methanol. A cost effective extraction unit of moderate capacity has been designed for the extraction of pure rutin on commercial scale.

Conclusions

Cultivation of *Eucalyptus* species in mid hills, as a rich source of rutin, is advantageous for the following reasons:

1. Pure rutin of USSRP grade or NF grade can be extracted from Eucalyptus leaves of two Species with less difficulty and low expenditure.
2. Raw material can be produced in large quantities.
3. It can be a good source of income for marginal growers by utilising their spare land.
4. Return per unit area is comparatively more.
5. The leaves of *E. macrorhyncha* and *E. youmanii* are used exclusively for the extraction of rutin. This is not so in *Sophora japonica* and Buckwheat.
6. *Eucalyptus* species can be grown as high density plantations whereas *Sophora japonica* trees are usually grown scattered.
7. It requires regular deployment of labour force to generate farm based employment for harvesting leaves from Eucalyptus plantations. The flower gathering period is only one month in case of *Sophora japonica*. In buckwheat also the harvest is made during a specific period only.
8. Besides rutin, the eucalyptus trees yield wood for fence posts and fuel.
9. These two species do not need good land for growing on commercial scale. Buckwheat on the other hand, should be planted in fertile soil and should receive liberal quantities of mineral fertilizers.
10. Dry leaves of the two Eucalyptus species have a ready market and are sold at ten-twelve thousands rupee per tonne. The leaves can be stored in a dry place in polythene bags for considerable period without any chemical change.

There is vast potential to increase the production of rutin from plant species of *E. youmanii* and *E. macrorhyncha* in the subtropical regions in Himalayan belt. They can be raised to produce rutin of high purity and foreign exchange of about crore of rupees can be saved annually. Small and marginal farmers can take up the cultivation of these exotic plant species in their spare land to generate additional income. Industrial units for extraction of rutin can be set up at central places to utilize the available raw material.

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Industrial Potential of Rutin Bearing *Eucalyptus* in Himachal Pradesh

B.L.Bradu and B.L.Kaul

Regional Research Laboratory,
Jammu.

R.Kapoor, N.K.Kalia and Y.S.Bedi

Regional Research Laboratory,
Extension Centre, Palampur (H.P.)

Introduction

The genus *Eucalytpus* (family Myrtaceae) embodying 600 species, is indigenous to Australia. Many species are reputed for timber, pulp, essential oils, rutin, tannins etc,·(Penfold and Willis, 1961). Their usefulness and wide adaptability have made them the subject of investigation and introduction throughout the globe. Two species namely *E. macrorhyncha* and *E. youmanii* constitute the important source of rutin (Vitamin P) in Australia. In mid - sixties the first introduction of *E. macrorhyncha* F.Muell in Nilgiris and Palani hills of South India was a success, yielding rutin 6-10 percent from leaves (Devar, 1969). No systematic effort was made, thereafter, to cultivate rutin yielding species except their introduction in Palampur and BaijNath areas of Kangra valley (H.P.) during 1974 (Bradu et al. 1982). *E. youmanii* has been a maiden introduction in the country. Based on the preliminary success of these species, the cultural practices were standardized. The tree crop plnations of the two species had been extended to various parts of the region and a large scale cultivation has been established during last more than two decades, forming single largest source of rutin in India. Under Palampur Baijnath

conditions, the average rutin content obtained from the leaves of *E. macrorhyncha* and *E. youmanii* has been 8.90 and 12.0 percent respectively. *E. delegatensis* is the only other rutin bearing species having comparatively less percentage (4-5%) Rutin commonly known as P factor (implying for permeabilus) or Vitamin P is the only flavone which is clinically established both as 3-rutinoside as well as its aglycone (quercetin), used for its therapeutic properties both in allopathic as well as Ayurvedic system of medicine. These two in combination with ascorbic acid are used to treat capillary bleeding due to increased capillary fragility. In cases where capillary fragility is concerned, the drug acts as an aid in the prevention of apoplexy and retinal haemorrhage, besides coronary thrombosis and purification of blood, retinitis and possibly rheumatic fever of haemorrhagic conditions. The drug has shown to provide protection against harmful effects of radiations (Stecher, 1960). Akamatsu (1931) studies the effect of rutin on the cardio-vascular system and found that the compound increased the stroke volume of both intact and isolated frog's heart. Ryan, (1955) found that the drug exhibit the properties of hyaluronidase, especially with ascorbic acid like hesperidin, which incidently, works as an oral contraceptive. As an analytical reagent quercetin can be prepared from it in a high state of purity by simple acid hydrolysis (Szarvas and Jarabin, 1959).

Rutin was first isolated by Weiss (1842) from *Ruta graveolens*. A number of plant species from different families have been found to contain rutin in various proportions in which some of the prominent species like *Sophora japonica* flowers (Leguminosae), *Fagopyrum esculentum* and *F. tataricum* leaves (Polygonaceae) are utilised as important source of the drug.

Botanical Features

The Australian tree species when grown undisturbed exhibit luxuriant growth under Palampur - Baijnath conditions in H.P. Comparatively among the two species, *E. macrorhyncha* exceed in height (approx 20-25 metres) than *E. youmanii* (18-20 metres). In *E. macrorhyncha*, bark dark grey, furrowed and fiberous; leaves lanceolate, acuminate, 8-12 cm. long; lower ones broader, thick and coriaceous, vein very oblique, prominent; calyx tube turbinate, the edge forming a prominent ring with the conical lid; anthers reniform, opening by divergent slits: Fruit depressed, globose, 4-6 lines thick; rim broad, convex. In *E. youmanii*, leaves are lanceolate - ovate, stalked, alternate, oblique, slightly falcate, coriaceous *E macrorhyncha* started flowering and fruiting in the ninth year of growth, whereas *E. youmanii* started flowering earlier in the seventh year. Seeds in both the species are viable ranged between 50-55% in *E. macrorhyncha* and 70-80% in *E. youmanii*.

Climatic adaptability

The two *Eucalytpus* species got well adapted to the sub-temperate climate of Palampur (Kangra valley) H.P., situated in the Himalayan foot-hills of Dhauladhar range at 1350 m altitude, 76.3° longitude, 32.6° latitude with an annual rainfall of 268cm. The maximum and minimum temperature during the year range from 30°C

(May) to 6°C (January). The established plantation in the terraced fields in the perimeter of 25-30 km. form the nucleus for its extension to different locations in the region.

Agro-technology

i) Soil and tillage

Eucalyptus is cultivated on fairly wide-range of soils but clay loam gives it a right foot- hold, being a tree species of longitudinal growth habit. As a forest tree species, it thrives in forest soil with soil pH around 6.5, that can be best suited for its successful cultivation. In terraced or plain fields, land should be ploughed to good filth and uniformly planked. In the slopy land, pits of 60 cm^2 size and 30 cm in depth are made at 1.25 - 2m spacing similar to forest planting. Well ploughed and planked land surface should be divided into manageable beds of 25-28 m^2 with proper irrigation channels.

ii) Nursery Raising

The preliminary sowing of seed in March-April revealed that young plants transplanted in mid-April to May were unable to tolerate heavy showers of Dhauladhar range during monsoons. The mortality was high due to damping off and around 15 percent saplings got afflicted with leaf spot disease. In order to cope up with the challenge of heavy death toll of March-April sowing during monsoons, post-monsoon sowing of seed is carried out in September. Seeds are sown in polythene bags (15×8 cm size), filed with soil and high proportion of organic matter. The germination is complete within a week to ten days. The germination rate is maximum at the optimum temperature range of 20-22°C. To improve the germination percentage, sometimes viable seeds are pre-treated with 50 ppm GA_3 for 24 hours before sowing. The seedlings with 3-4 pairs of leaves are transplanted by October end. Transplanting is carried out with great care and the seedlings are taken out alongwith the ball of earth after splitting apart the polythene bags. The ridges made are 15 cm. high and 45 cm. wide and the saplings are spaced 1.25m apart. The growth continues till November and dormancy is accorded in December when the atmospheric tempeature gets lowered to 2-3°C. With the onset of spring the spurt in growth is visible which is further accelerated during monsoons. Even in case of excessive monsoon did not lead to any appreciable mortality and by September the plants in general attained a height of upto one metre. About 5- 600 gms. of viable seed is required to stock one hectare.

(iii) Irrigation

The tree crop requires assured irrigation in the early period of growth in plain area cultivation when tree saplings are transferred to the field. Three to four irrigations are required during the period from April to June. The crop in the early stages of growth is sensitive to water-logging. In case of slopy field or planting in

the hill, pre-monsoon sowing of seed can take place, but transplanting should be delayed till monsoons so that the crop get established in rain-fed conditions and no-risk factor of water-logging is encountered.

(iv) Interculture and Weed Management

Weeding and light hoeing is necessary in the early stages of growth. The interculture operations continues during first two years after transplanting. Weed competition is lowered when the crop attains maturity in the third year and ready for first harvest. Now onwards till seventh year the weed management and inter-culture operations should be carried on after every harvest.

(v) Nutritional requirement

The soil condition can be improved by liberal application of farm yard manure (F.Y.M.) at 10 tonnes/ha, spread uniformly at the time of field preparation. Any sign of chlorosis in the seedlings after transplanting could be corrected by weekly sprays of 0.2 percent urea. This could be supplemented by enriching the soil with a basal dose of 50N, 50P, 20K kg/ha depending on soil status as revealed by physical and chemical analysis. Under irrigated conditions, the fertilizer application of urea at 312 kg N/ha in two equal split doses during the growing periods of each harvest in the two *Eucalyptus* species viz march and July months, has resulted in the leaf biomass increase upto 22% over control. Under rain-fed conditions, six foliar applications of nitrogenous fertilizer (Urea) at 0.3 percent concentration distributed proportionately during the growing period of two years's harvests has resulted in the gain of leaf biomass upto about 50% over control. Effect of n-triacontanol as foliar application on the vegetative growth revealed that 10 ppm concentration of the hormone in four sprays annually distributed between the two harvests has staged an increase of 51-53% of leaf biomass.

(vi) Harvesting

The two *Eucalyptus* species attain maturity in the third year when growth level reaches upto six feet height. Coppicing practice has been adopted at four feet height to obtain the leaf yields. Yearly two harvests are taken, first synchronises in the pre- monsoon period i.e. May - June and second in October - November in the post-monsoon season. The schedule for two harvests in a year continues till seventh year based on the economic yields of leaf-biomass and rutin. The annual fresh herb yield exhibit increase in first harvest than in second and the increase being 4-5% in *E. macrorhyncha* and 7-8% in *E. youmanii*. The overall herb yield in *E. macrorhyncha* showed an edge over *E. youmanii*.

(vii) Leaf and Rutin yields

The average annual fresh leaf yields in *E. macrorhyncha* are 4.5, 5, 6.5, 8.5 and 10 tonnes in the consequent years from third to seventh year. In *E. youmanii* the yields are 4, 5, 6.5, 8.5, 9.7 tonnes respectively in the third and subsequent years.

Average rutin yield *E.macrorhynchya* = 6.8-11.1% (8.9 %)

E.youmanii = 10.4-15.7% (12.0%)

(viii) Sapling hygiene

The two species are quite susceptible to termite and leaf sucker attack which can be checked by prophylactive doses of Aldrin or BHC powder incorporated in the soil at nursery or transplanted stages. Collar rot and lealf spot diseases often occur in the nursery plantation caused by *Sclerotium* spp. and *Alternaria* spp. respectively. Collar rot can be controlled by a spray of 0.4% Bevistin and *Alternaria* by Diathene M-45 0.20%.

Extraction of Rutin from leaves

Rutin is highly soluble in a number of organic solvents like methanol, ethanol, pyridine etc. but solvent extraction of leaf material gives a product which contain unwanted substances alongwith rutin, which is highly coloured besides being uneconomical. Though rutin is poorly soluble in cold water (1 gm. in 8 litres of water), but is fairly soluble in boiling water (1 gm in 200 ml of boiling water). Also water does not dissolve most of the undesirable substances. The plucked dried leaves are reduced in size using grinding mill and digested in boiling water, followed by filteration. The residual leaf material is washed, filtered and added to main filtered material liquor. Crystallisation of rutin takes place on cooling and pure rutin is obtained after filteration. Re-crystallisation can be done if needed. The rutin cake obtained is dried, powdered and packed.

Project Economics

(1) Capacity

(a) Dry herb yield of *E. macrorhyncha*

3rd year	=	2.2 - 2.5 tonnes/ha
4th year	=	2.5 - 2.6 " "
5th year	=	3.2 - 3.5 " "
6th year	=	4.0 - 4.5 " "
7th year	=	5.0 - 5.2 " "

(b) Dry herb yield of *E. youmanii*

3rd year	=	1.8 - 2.0 tonnes/ha
4th year	=	2.2 - 2.5 " "
5th year	=	3.0 - 3.5 " "
6th year	=	4.0 - 4.2 " "
7th year	=	4.5 - 5.0 " "

(ii) Project cost:

Ist year = Rs. 20,000 - 22,000/ha

Second year = Rs. 15,000 - 16,000/ha

Third year = Rs. 12,000 - 13,000/ha

(onwards till 7th year).

(iii) Cost of Production

a) *E. macrorhyncha* (dry herb)

3rd year = Rs.5.20 - 5.40/kg of dry herb

4th year = Rs. 5.00 - 5.20/kg " " "

5th year = Rs. 3.70 - 4.00/kg " " "

6th year = Rs. 2.80 - 3.20/kg " " "

7th year = Rs. 2.50 - 2.60/kg " " "

(b) *E. youmanii* (dry herb)

3rd year = Rs. 6.50 - 7.20/kg of dry herb

4th year = Rs. 5.20 - 5.90/kg " " "

5th year = Rs. 3.70 - 4.30/kg " " "

6th year = Rs. 3.00 - 3.20/kg " " "

7th year = Rs. 2.60 - 2.80/kg " " "

(iv) Profitability

(based on sale of dry herb at Rs. 20/- kg)

(a) *E. macrorhyncha*

3rd year = Rs. 32 - 37,000/- ha

4th year = Rs. 38 - 39,000/- ha

5th year = Rs. 52 - 57,000/- ha

6th year = Rs. 68 - 77,000/- ha

7th year = Rs. 88 - 91,000/- ha

(b) *E. youmanii*

3rd year = Rs. 24 - 27,000/- ha

4th year = Rs. 32 - 37,000/- ha

5th year = Rs. 49 - 57,000/- ha

6th year = Rs. 68 - 72,000/- ha

7th year = Rs. 78 - 87,000/- ha

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*Cultivation of Buckwheat in the Plains as a Raw Material for Rutin

Ajit Singh

Flat A/6, 4/40 Wazir Hasan Road, Lucknow - 226 001

Introduction

THE name buckwheat (*Fagopyrum spp.*) is confusing to many, since it seems to convey its relationship to the common wheat, which is not true. The name is probably a modification of "beech wheat" (German Buckweizen) from the resemblance of "seeds" to beechnuts and the fact that flour is prepared from the seeds. Whereas, common wheat is the true cereal of the family *Gramineae*, buckwheat is classified as pseudo-cereal belonging to an unrelated family *Polygonaceae*. The common buckwheat was not cultivated in ancient times as there is no Sanskrit name for it. It came to Europe through Tartary and Russia in the middle ages. In a recent archeological dig of the 11th to early 13th century in Bilyar (USSR), seeds of buckwheat were found in the cultural layer (Tuganaev and Frolova, 1975). Yamanaka *et al.*, (1992) after pollen analysis of the Holocene deposits from the Okoumire in the central part of the Kochi Plan observed that buckwheat cultivation possibly began during the early or middle Jomon Age (About 5000-7000 years ago). Buckwheat is believed to have originated in Central Asia as it is widely cultivated as a main or subsidiary food plant in several parts of the world, viz. USA, Canada, USSR, Europe, Japan, Korea, Spain, South Africa, India and Nepal. In India, it is grown almost entirely as a food crop in the temperate parts of the Himalayan ranges and the hilly areas of Tamil Nadu (Harbhajan Singh, 1961).

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*Revised and updated.

This plant received attention in 1946, when Couch *et al.*, (1946) reported that it is the most promising and economical source of rutin. The leaves and flowers are rich in rutin. Krause (1976) obtained rutin from the cotyledons of *F. esculentum*. Udo *et al.*, (1973) observed that rutin production was favoured by high temperature. Rutin has been shown to afford protection against harmful effect of X-rays, indicating that it may be of use to persons exposed to dangerous atomic radiations. Certain bioflavonoids, which are natural non-toxic food substances from plants, like rutin from buckwheat, prevent the oxidation of ascorbic acid and seem to protect the endothelium when given with Vit. C. Clemetson and Alan (1976) suggested that all Vit C tablets should be combined with these flavonoids. A simple method for determining rutin in buckwheat flour by HPLC was developed by Ohara *et al.*, (1989).

There are two important cultivated species, viz. *Fagopyrum esculentum* Moench and *Fagopyrum tataricum*. Gaertn which yield rutin. The leaves of *F. tataricum* are narrow and arrow-shaped, flowers are small, having inconspicuous light green sepals which distinguish it from *F. esculentum* which has thicker and broder leaves and conspicuous pinkish-reddish flowers. Tahir & Farooq (1989) have detailed morphophysiological characteristic of *Fagopyrum* species grown in Kashmir.

Scope and Cultivation in the Plains

With the growing importance of rutin as a medicine, the crop gained some importance. Being essentially a crop grown at higher elevations (1200m), efforts were made to cultivate the crop at Jammu (300m) in 1966 Both the sepcies grew well, but *F. esculentum* with a higher leaf yield has a lesser rutin content (3 to 4 9%) as compared to *F. tataricum* (6 to 6.6%) *F. tataricum* was preferred for the production of rutin (Singh, 1967).

Cultivation

The land is thoroughly prepared and levelled because *Fagopyrum* as a crop is susceptible to water-logging and is a poor competitor with weeds. A thorough initial preparation helps in cutting down costs on subsequent ploughings as it is a short duration crop of 35 to 48 days, and from early September to early December atleast two crops can be grown on the same piece of land. It can be grown on light and rich soils, avoid water logged conditions. The seed rate of the crop for the production of leaves for rutin is between 55 to 65 Kg/ha, while for the production of seeds it is about 35-40 Kg/ha. The crop attains a height of 65 to 70cm, leaves contain 88 to 90% moisture, sundrying is slow, leading to loss of rutin. Thus, artificial rapid drying of the leaves is necessary. The period between bloom and seed setting is very short, a close observation is required to enable harvesting the crop at the proper stage. Being a photosensitive crop, the flowering is very erratic and plants as small

as 6 to 8cm can be seen in the flowering stage. 100 Kg of fresh herb on an average yield about 10 Kg of dry material (Singh, 1967).

A seeding rate of 30 to 45 Kg/ha ensured optimum yield of grain in most years (Gubbels & Campbell, 1986). Photosensitivity was established after subjecting the seeds to various photoperiods (Singh & Mall, 1977), Growth performance of the crop has been reported by (Tahir & Farooq, 1990; Prasad *et al.*, 1991; & Koblev, 1992).

Cultivars of buckwheat *Fagopyrum esculentum* Moench in Japan are classified in two ecotypes; summer and late summer types. The summer types are distributed in the high latitude (37- 45°) where as late summer types are in the region of low latitude (31-34°). It is reported that the summer type cultivars are non-sensitive to day length unlike the late summer types (Minami & Hyoji, 1986). Studies on the reactions of various buckwheat varieties on the changes of water supply or on the effect on soil, air and air, soil-air drought show that soil-air drought had the strongest negative effect on plants leading to partial death of buds and fruits at the stage of their formation (Lakhanov, 1992).

Sowing Period

The best sowing time of the crop for obtaining maximum production of leaves for rutin in the plains of India appear to lie between early September and end of October. Crop germination is complete within a week for crops sown during early September and October. It was.observed that the crop sown between December and January showed delayed germination and was badly affected by frost. In the February sown crop germination was slow (10-15 days), fresh herb yield was poor, but the crude rutin obtained was 6.0 to 6.6%. The crop sown in March germinated within 4 to 5 days, but the warm season during March and April was not very conducive for growth. In spite of frequent irrigations the crop presented a burnt up appearance, yield of fresh herb was poor. If initial preparation of the land is good, there is no need for interculture or else one weeding is required. One irrigation after 15 days of sowing meets the irrigation requirement of the crop (Singh, 1967).

Fertilizer

Taylor and White (1950) reported that on fertile soils only P was needed and on less fertile soil supply of N was also necessary. They recommended a dose of 24 lb N and 48 lb P/acre. It was significant to observe that percentage of rutin did not increase. Strong (1973) suggested that utilization of applied phosphorus was aided by the high efficiency of *Fagopyrum* roots in absorbing P from high concentrations. Krause and Reznik (1976) observed that plants grown under P and N deficiency accumulated flavonols to a higher extent than those receiving a complete set of nutrients. Greater phosphorus uptake by buckwheat was associated with its ability to acidify the rooting medium (Mclachlan, 1976). Chakmak and Horst (1986) report that increasing P supply resulted in severe Zn deficiency symptoms (interveinal

chlorosis). Zn deficiency markedly increased uptake and translocation rates of P. The concentration of K and Mg in leaves were not affected by Zn deficiency.

Yagodin and Sablina (1986) showed that application of cobalt essentially increases the content and gather of rutin in buckwheat. Pre-sowing treatment at 0.02% solution of $CaSO_4$ gave the highest content of rutin. The application of cobalt increases the activity of polyphenol-oxidase and peroxidase which participate in rutin biosynthesis. The elimination of cobalt from the nutrient mixture resulted in a sharp decrease in rutin content. On leached Chernozem soils Yagodin *et al.*, (1987) reported that the amount of N; P&K in the vegetative mass of buckwheat is more subject to variation than their amount in grain, while Yagodin *et al.*, (1989) recommended pre-sowing application of cobalt if more than 60 kg of N was applied per hectare. The yield and grain quality both were increased due to higher amount of proteins.

Labude (1991) has presented a new theoretical approach to fertilization based on the view that knowing the ratio of elements in crops at the flowering stage can be used in determining the appropriate ratios of fertilizer components in plant nutrition and fertilization. The crops studied were buckwheat, oats, potato, rye, spring barley and winter wheat.

Thus, the application of fertilizers for this short duration crop of 35 to 50 days has to be very timely, so that the crop could best use the nutrients applied within a short time.

Harvesting

The best stage for harvesting the crop is when the plants begin to bloom (35 to 45 days of sowing). If harvesting is delayed beyond this period, there is a sharp decline in the rutin content from 5.14 to 3.83% (Singh, 1967). For obtaining maximum rutin yield from *Fagopyrum tataricum* in Gujarat, Patel & Kayan Sundaram (1984) suggest that leaves alone may be harvested during initiation of seed setting which occurs at about 50 days after sowing and the leaves dried for about 36 hours to effect 85% moisture loss.

Cha *et al.*, (1989) report that Growing degree days (GDD) required from planting to flowering time was 440- 470°C and GDD required from flowering time to optimum harvesting was 630°C in July 11 planting and 480-540°C in July 21 and 31st plantings.

Breeding

Alekseeva *et al.*, (1988) observed that of radical importance in buckwheat breeding for immunity to fungal diseases is the number of stomata in the epithelial tissue of the upper epidermis of leaves, in breeding for resistance to viral diseases – hairiness of plant organs, while Takahata (1988) observed that maintenance of valuable genotypes of *F. esculentum* was difficult owing to heterostylic self-incompatibility. To establish a propagation system, he developed a procedure for plant regeneration

from immature infloresence culture. Buckwheat does not have a strict centre of genetic diversity, but the highest within population variability was found in the region from Southern China to Nepal (Ohrushi, 1988). Singh *et al.*, (1989) made a genetic evaluation of 12 promising lines of *Fagopyrum*. Rutin yield showed significant positive correlation with number of leaves/plant, leaf yield/sq. m. (g, dry mass), herbage yield/sq. m (kg) and plant height (cm) respectively. Besides, leaf and herbage yield also had a significant regression (b) with rutin yield, therefore these characters can form a good selection index in improving rutin yield of *Fagopyrum*.

Lakhanov (1991) found that response of buckwheat cultivars to water supply is genetically determined by the level of development of physiological mechanism of resistance and their repair capacity. Manzkulin *et al.*, (1991) showed that plant transition from disploidy to tetraploidy had no influence on chloroplast volume and ultra-structure. Fesenko & Martynenko (1992) have discussed the evolutional aspects of breeding as a process of transformation of ontogenetic development correlation with buckwheat and wheat as examples. Guan and Adachi (1992) observed that diploid genotypes which are less sensitive to unfavourable conditions, should be considered for use as a breeding material in buckwheat.

Diseases

A number of diseases are known to occur on the crop, *Sphace lotheca fagopyri* (Fries, 1855), *Puccinia fagopyri* (Barclay 1890), and *Septoria polygonicola* (Sydo & Butler, 1916) have been reported on *F. esculentum*. Thakur (1974) reported for the first time the occurrence of *Alternaria alternata* (Fr.) Keissler on *F. tataricum* crop grown in Jammu. Morrall & Mckenzie (1975) reported that in Canada the most prevalent disease of *Fagopyrum esculentum* was botrytis stem rot (*Botrytis cinrea*). They also recorded *Rhizoctonia* spp. and *Sclerotinia sclerotiorium* for the first time. The incidence of harmfulness of buckwheat viral blight on *Fagopyrum tataricum* and *F. cymosum* was shown and *Bacilliform* virus was found to be the causative agent of the disease. Immune sps., less affected cultivars and mutants were revealed for the 1st time (Alekseeva, *et al.*, 1988). Zimmer *et al.*, (1990) observed stunting of buckwheat seedlings for the first time in 1982. These symptoms may be the result of seed-borne systemic infection by the downy mildew pathogen *Peronospora ducometi*.

Uses

Traditionally in India buckwheat grain is used as food mostly by the hill folk, buckwheat flour is taken during religious fasts. Buckwheat flour is a major composition material of 'Soba' noodle which is a very favourable traditional dish among the Japanese. The use of gamma irradiation on buckwheat flour and buckwheat food products like noodles has increased their shelflife, and not effected the characteristic flavour and texture of 'Soba' noodle (Muramatsu *et al.*, 1988; Ohinate *et al.*, 1988).

Fornal *et al.*, (1986) suggest that starch quality significantly determines the expansion coefficient, density, and physico-chemical properties of the extruded starch mixture and the unique properties of buckwheat starch were most important. Hong *et al.*, (1987) report that the inhalation of buckwheat flour as well as injestion of buckwheat food can induce bronchial asthma, while Soyer & Margit (1987) report about a patient with allergic urticaria after intake of buckwheat food.

Young buckwheat plants have been used as vegetable and medicinal crop for a long time because of its rapid growth rate, high protein and rutin content and a more favourable ratio of leaves to stems than mature plants (Choi *et al.*, 1991).

The leaves of *Fagopyrum tataricum* on stem distillation yielded essential oil (0.36%) with the following constituents: α-pinene, 8.76%; d-limonene, 8.6%, α-terpenol, 15.8%; bornylacetate 17.3%; α-thujene 14.0%; α-terpenolene 2.0%; myrcine, 7.1%; camphene, 5.1%; methyl chavicol 9.2% eugenol, 2.5%; unidentified alcohol 2.2% (Samaiya and Saxena, 1986).

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*ALOE

M.K. Raina

Herbal Research Centre
Lupin Laboratories Limited, Bombay

Introduction

THE term 'Aloe' as described in Pharmacopoeia and used in medicine stands for the dried juice of leaves of *Aloe perryi*, Baker, also known as Socotrine aloes; or of *Aloe barbadensis* Miller (*A.vera* Linn.) known as Curacao aloe or Aloe ferox Miller and hybrids of this species with Aloe africana Miller and Aloe spicata Baker, known as Cape aloes[1-3].

Aloe genus (Family: Liliaceae) comprises about 200 species, indigenous to East and South Africa. A number of species have been introduced into India which grow in varied climates and different types of soils. The fleshy leaves in rosettes are usually prickly at the margin. A yellowing coloured juice flows out from the cut leaves, which is collected and concentrated to make commercial drug aloes. The nature of commercial drug aloes depends upon the species from which it is prepared and the method adopted for concentration. Sun dried aloes and concentrated over low flame makes amorphous, opaque type called hepatic or livery aloes, whereas juice concentrated rapidly over strong fire, gives semi-transparent type called glossy or vitreous aloes[1-3]. Four types of aloes are official in Indian Pharmacopoeia[2]. These are Curacao or Barbados aloes, Socotrine aloes, Zanzibar aloes and Cape aloes. These occur as dark brown opaque masses, almost entirely soluble in 60% alcohol.

Aloe is derived from Arabic word alloeh or the Hebren halel meaning a shining bitter substance. Vera is from Latin Verus meaning true, Barbadensis refers to the Barbados islands : ferox is from the Latin meaning wild or ferocious; africana refers to the habitat of the plant and spicata refers to the flowers in spikes[4].

Aloe vera is a perennial plant with multiple tuberous roots and many fibrous supporting roots penetrating into the soil. The plants do not have true stem but produce bloom stalks. Flowers vary from yellow to rich orange in colour. The plants generally grow close to the ground in typical rosette shape. The plant has strongly cuticularized leaves, having spiny margin with thin walled tubular cells[4]

Aloe is known by different names in different languages of the country as follows[1-3,5-9]:

Bengali	-	Ghrit kumari
English	-	Aloes
Gujarati	-	Kunvar
Hindi	-	Ghikanvar
Kannada	-	Katthaligida, Lolesara, Kumari
Kashmiri	-	Musabbar
Malayalam	-	Kattavala
Marathi	-	Elwa, Korpad, Kunvar pata
Tamil	-	Kattazhai, Sirukattashai, Chirukattali
Telugu	-	Manjikattali, Chinnakalabanda
Urdu	-	Ghiqwara

Uses

In Africa, slukaris in the Congo rub their bodies with mucilage from leaves to reduce perspiration and thereby eliminate human scent. This helps in masking their scent to the prey. The cut leaves of *A.saponaria* are tied on fleshy surface of wounds by some tribes of South Africa for early cure. It has been recorded that Indians used the pulp of *A.barbadensis* mixed with burnt alum for healing country sore eyes. The pulp is also valued as a cooling application on inflammatory joints. To relieve headache, a small piece of sliced leaf is tied to the forehead. Some Africans use the cold infusion of leaves of *A.saponaria* for hair growth. The leaves of *A.variegata*, infused in brandy or liquor is taken to relieve haemmorhoids. During epidemics of cold in Sutos, all people in the village bathe publicly in an infusion of *A. latifolia*[8,10].

The parts used in indigenous system of medicine are expressed as dried juice of leaves and pulp. Aloe is reported to be stomachic tonic in small doses whereas in large doses it acts as purgative and indirectly emmenagogue and anthelmintic. It has been a favourite remedy for intestinal worms in children. In the form of a lotion, it is recommended in catarrhal and purulent ophthalmia. Aloe dissolved in spirit, is used as a hair dye to stimulate hair growth. A sweet confection prepared

from the pulp of the leaves is given in piles. Pulp mixed with honey and turmeric is recommended in coughs and colds. The juice of the leaves is useful in painful inflammations and chronic ulcers[5,6].

More recently the use of aloe gel in radiation burns has been advocated for relief from pain and itching and to keep down keratosis and ulceration, thus slowing any possible change towards malignancy[11].

The Ayurvedic literature describes the actions and uses of aloe as follows[5]:

Bhedinee (purgation) netrya (beneficial to the eyes), rasayance (rejuvenator), balya (muscular strength), vrishya (that which promotes virility), *Gulmaghna* (indicated in the treatment of tumour like conditions of the abdomen), vishagna (antidote for poisons), pleecharogaghna (indicated in splenomegaly). *Yakritvriddhihara* (that which cures hepatomegaly), kaphajwarahara (that which cures fever caused by kapha), grantighna (that which cures glandular enlargement), *agnidagdhashamanee* (that which relieves the effect of burns), kushtaghna (indicated in skin diseases), shwaasaghna (antispasmodic, anti-dyspnoic).

Aloe is commonly used as an external application on inflammed painful parts of the body. Aloe was known to the ancients having been cultivated in the island of Socotra as early as at the time of Alexander the Great. It has been employed for eczematous skin conditions in China, India and Tibet. The wounds are quickly cicatrized and healed when covered with a small piece of Aloe. Fresh Aloe leaf gel is also reported to be of value in the treatment of X-ray reactions[1].

Aloes Powder and aloin are official in I.P. as a purgative. Aloin is a mixture of crystalline compounds obtained from Aloes. It varies in chemical composition and in physical and chemical properties depending on the variety of aloes from which it is obtained. It is generally yellow coloured microcrystalline powder with a faint odour of aloes and intensely bitter taste. On exposure to light and air, it turns dark. Aloin is water soluble as well as alcohol soluble (90%). It is sparingly soluble in chloroform, solvent ether and in benzene[2].

The United States Dispensatory refers to the use of Aloe gel as early as 2,300 years ago. It is repeatedly referred in the Holy Bible and was extensively used in India as long as in B.C. 400 [12].

A large number of research workers have published reports of their findings on the use of Aloe vera leaf in various skin conditions. The healing of abrasions of human skin showed definite improvement by application of fresh *A . vera* leaf. A five week treatment of *A. vera* in patients of severe X-ray dermatitis showed complete regeneration of the skin on the scalp and forehead, new hair growth, restoration of sensation and no scarring 12,13. *A.vera* gel was found useful in the treatment of sequelae to radiation therapy. Excellent results were obtained on the use of this gel in palmer eczema, ulcers on amputation stumps, ulcers of advanced mammary carcinoma, poison ivy and burns. Application of fresh aloe pith relieved

pain, burning and itching, had anti-septic action and stimulated rapid granulation and formation of new tissues[13].

It was suggested that solution of *A. vera* might be incorporated into periodontal dressing. Studies were conducted on the use of *A.vera* gel in the treatment of ulcers and certain other dermatoses in men, like chronic leg ulcers, seborrhea, acne vulgaris, alopecia and alopecia areata. The results showed stimulating effect on the healing of chronic leg ulcers, stimulate hair growth and drying of seborrheic skin. The action is believed to be due to the presence of mucopolysaccharides in the gel which may be aided by enzymatic removal of necrotic tissue[12].

The therapeutic effect of *Aloe vera* have been examined in preventing progressive dermal ischaemia caused by burns, frostbite, electric injury, distal dying flap and intra-arterial drug abuse. The studies indicated that Aloe has enormous therapeutic potential as it penetrates tissue injury, relieves pain, is an antiinflammatory and dilates capillaries and increases the blood supply to the injured area by inhibiting TxA_2 and maintaining the PGE_2 and PGF_{2a} ratio without causing a collapse of the injured blood vessels[14].

Extracts of *Aloe vera* possess activities which reverse the degenerative skin changes seen with aging by stimulating the synthesis of collagen and elastic fibers, thereby restoring towards normal the regenerative/degenerative equilibrium[15].

Cultivation

The propagation of Aloes is done through root suckers. The plant can be cultivated in dry climatic conditions, in poor soils without much care. The root system of this plant is shallow and does not penetrate deep into the soil. A mixture of nitrogen, potassium and phosphorous should be used as manure and mixed up in soil near the root system gently. Since the plant is not water loving, water should not be allowed to lodge near the plant[3].

Recently leaf spot disease on *Aloe vera* growing in Bhagalpur and its surrounding areas has been found to be caused by *Alternaria alternata* and *Fusarium solani*[16]

Studies have indicated that irrigation has the greatest effect on the gel composition of *Aloe vera*. Hence control of irrigation could aid in standardizing the gel composition[14].

Tissue Culture

Earlier research workers have reported successful callus formation from the root of *Aloe saponaria*, stamen of *Aloe bellatura* and leaf of *Aloe barbadensis* in illuminated conditions However as tissue culture from *Aloe aborescens* Miller var. natalensis (Kidachi aloe) had not been reported, two projects were planned using the same.

1. To determine the culture condition in which callus of Kidachi aloe can synthesize active components of Aloe such as carboxypeptidase or aloin more effectively.
2. To obtain a more useful hybrid plant by cell fusion and regeneration of plants.

The experiments showed that callus was induced when incubated in the dark at 25°C for about 30 days. The frequency of callus formation was in the order of superior stalk > middle stalk > inferior stalk. No callus formation was induced from the leaf. When the Murashige-Skoog (MS) basal agar medium was supplemented with 3% sucrose, 0.1- 0.5 μm Kinetin (a plant growth regulator) and 10-50 μm α-naphthalenacetic acid, callus formation occurred at an indicence of 20-52%. When the medium was supplemented with 3% glucose and 10% alpha modification of Eagle's medium, callus formation was promoted, but protein and aloin concentration, and carboxypeptidase activity in the Aloe callus decreased more than those observed in the sucrose-containing medium[18].

Pharmacognostic Studies

The plants bear rosettes of large succulent, subulate leaves, flat or slightly concave on the upper surface and strongly rounded on the lower, sessile with a strong spine at the apex and smaller ones along the margins. They bear spikes of yellow to red flowers[19].

The vascular bundles in the leaf are isolated and form a line parallel with epidermis within the mesophyll. Each bundle has a pericycle formed of large thin walled cells filled with yellowish fluid. These cells are slightly elongated[19].

Socotrine aloe has a pasty, semi-liquid consistency, opaque with brownish-yellow colour. It is dried at a gentle heat to remove moisture when it turns to hard, dark brown mass. It has a characteristic unpleasant odour and extremely bitter, nauseous taste. It is entirely soluble in alcohol.

Zanzibar aloe is usually hard in liver-brown colour with characteristic but not disagreeable odour and bitter taste.

Cape aloe occurs in dark reddish-brown or nearly black coloured mass which has a clean glossy fracture. It has a distinct sour odour.

Curacao aloe occurs as yellowish to reddish-brown or chocolate-brown and breaks with a dull waxy even fracture[19].

The above four kinds of aloes can be distinguished, when in powder form, by microscopical examination mounted in lactophenol. Lactophenol has an advantage over any other oil or liquid paraffin or glycerine in that it brings about a gradual solution of the particles and the crystals become rapidly and clearly evident. Cape aloe appears as transparent, brown, irregular and angular fragments; Curacao aloe shows fragments composed of innumerable minute slender prisms or needles; Zanzibar aloe shows irregular lumps with embedded nodular mass; whereas So-

cotrine aloe consists of fragments of fairly large prisms grouped irregularly into mass[4,19].

Commercial aloes occur both in crystalline and amorphous forms. The crystalline aloes respond to test for Curacao aloeo and the amorphous ones to tests for Cape aloe. The two varieties are easily distinguishable under the microscope by mounting in cresol and using polarized light. The crystalline aloin is insoluble in cresol and shines brightly on a polarized light against dark field. The amorphous aloin dissolves rapidly and is invisible under polarized light against dark field. The amorphous aloin has one third the cathartic potency of crystalline aloin[4,19].

Chemical Studies

Aloin is the principal active constituent of aloes which is a mixture of glucosides. Barbaloin is the main glucoside in aloin which is water soluble. Besides this, aloin contains isobarbaloin, β-barbaloin, aloe-emodin resins, etc.[3]

The percentage of barbaloin present in different types of aloes varies to a great extent. Estimation of barbaloin in Indian aloes obtained from *Aloe vera* var. officinalis shows that the Indian species contained less quantity (3.8%) as compared to Curacao aloe which contains 22.1%[20].

The following chemical constituents have been reported to be present in different proportions : aloin, aloe-emodin, aloetic acid, homonatalion, aloesin, aloesone, emodin, chrysamminic acid, chrysophanic acid, apoise, galacturonic acid, calcium oxalate, choline, choline salicylate, saponins, uronic acid, sugars, mucopolysaccharides, 7-hydroxy chromone, coniferyl alcohol, glucosamines, hexuronic acid, amylase, aliinase, etc. Many of the above constituents have been isolated in pure form[13].

The lyophilized *A.vera* juice on neutron activation analysis was found to contain calcium (4.7%), sodium (1.43%) potassium (6.6%), chloride (12.2%) and manganese (0.01%)[21].

The main constituent of all the varieties of aloes is the crystalline substance, barbaloin (aloe-emodin anthrone C-10 glucoside). In Curacao aloe, barbaloin is accompanied by isobarbaloin. Socotrine and Zanzibar aloes do not contain isobarbaloin whereas Cape aloe contains traces only. The crystalline aloin is accompanied by an amorphous aloin, beta barbaloin. The latter is particularly abundant in Cape aloe. The other main constituents of aloes are resin and aloe-emodin, a hydrolytic decomposition product of barbaloin[19].

Studies on the leaves of *A. vera* grown in Egypt showed the presence of free combined anthraquinones, carbohydrates and/or glycosides. Barbaloin, chrysophanol glycoside and aloe-emodin were isolated and identified. The mucilaginous parts of the leaves were found to contain sugars glucose, galactose, mannose and galacturonic acid[22].

The rhizomes of *A. saponaria* yielded phenolic constituents[23]. From the leaves of *A. barbadensis*, free amino acids, free monosaccharides and total saccharides released upon hydrolysis, sterols and triterpenoids have been isolated[24].

An analysis of the *A.arborescens* leaves during different seasons of the year revealed the presence of highest content of total nitrogen (1.47%), protein nitrogen (1.22%), total protein (9.54%) and real protein (7.63%) in leaves sampled in April; the highest content of aloin (4.31%) and free amino acids (798.17 mg per 100g leaves) in July. Samples collected in January had the lowest amount of components. The leaves of upper regions were comparatively poor in chemical constituents[25].

Analysis of the dry mass obtained from aqueous extract of fresh leaves of three year old *A. arborscens* showed 6.3% total protein, 3.47% non-protein substance containing nitrogen and 0.6% free amino acids[26].

A carboxypeptidase was partially purified *Aloe arborescens* Miller var. *natalensis* Berger on a scale suitable for pharmacological studies. The enzyme had a broad specificity against various synthetic peptides, being capable of splitting C-terminal proline. Its activity was inhibited almost completely by di-isopropylfluorophosphate, strongly by transition metals, such as Fe^{3+}, Hg^{2+} and Cu^{2+}, and moderately by sulphydryl reagents. These results indicate that Aloe enzyme is a serious carboxypeptidase and appears to contain a sulphydryl group that may be involved in its inactivation[27].

Pharmacological and Clinical Studies

A. indica showed a significant increase in the fertility rate of experimental rabbits receiving 60 mg/kg or more of aloe. There was a significant increase in the litter size of these animals.

The aqueous extract *A. littoralis* inhibited the growth of Staphylococcus and *Escherichia coli*, while alcoholic extract of the same did not show any measurable inhibition.

A. vera, according to all available data does not cause or contribute to toxicity in humans or higher animals in gel form or its various extracts. Aloin, however, can be irritating to the skin in higher concentrations[12].

A number of cosmetic preparations containing aloe extracts in varying proportions are available in the market. Safety data on the various aloe extracts are also available. *A. vera* extracts have been considered safe when used near the eye, on the skin or when ingested in a single dose[13].

A. vera juice in the form of an ointment in vaseline has been found to hasten healing of wounds of thermal burns and radiation injury in albino rats. In addition to this, hydroxyproline and mucopolysaccharide contents were increased significantly in *A. vera* treated animals[29].

The antifertility effect of various extracts of leaves of *A.barbadensis* has been studied in rats. Alcoholic and water extracts showed 85% reduction in fertility while benzene and petroleum ether extracts showed significant reduction. The chloroform extract showed much less reduction in the number of young ones produced by each female rats[30].

Intravenous injection of extract of *Aloe barbadensis* was found to increase hepatic bile flow in anaesthetized dog[31].

Aloctin A was isolated and purified from *Aloe arborescens*. It showed many biological and pharmacological activities. i.e. *Haemagglutinating* activity, Cyto-agglutinating activity, Mitogenic activity of lymphocytes, Precipitate forming reactivity with alpha$_2$-macroglobulin, Complement C3 activating activity.

Inhibition of heat - induced haemolysis of rat erythrocytes, Antitumour effect, Anti-inflammatory effect and inhibition of gastric secretion and gastric lesions[32].

The effect of Aloctin A treatment derived from *Aloe arborescens in vivo* and *in vitro* on the immune response of murine and human lymphoid cells has been studied[33].

Lectins isolated from the gel portion of leaves of *Aloe barbadensis* were found to have strong haemagglutination and mitogenic activities[34].

Carbopeptidase was partially purified from Kidachi aloe (*Aloe arborescens* Miller var *natalensis* Berger) by an FPLC system, and was administered intravenously to female ICR mice with inflammation. The enzyme preparation exhibited significant analgesic effects and inhibited vascular permeability in the abdominal region. It also revealed an antithermal burn action on the hind paw, when administered to female Wistar rats intravenously[35].

The juice of *Aloe arborescens* was found to have both suppressive and healing substances and is useful in the treatment of gastric ulcer[36].

Two different components were separated from *Aloe arborescens* and these were found to exhibit hypoglycaemic activity in spontaneously diabetic and normal mice[37].

Inhibitory effects of freeze-dried *Aloe arborescens* whole leaf powder on induction of preneoplastic glutathione S-transferase positive hepatocyte foci GST P$^+$ were studied in male rats. It was found that 30% Aloe exerted inhibition of the promotion and possibly of the initiation stage of hepato-carcinogenesis[38].

Clinical studies with Aloes Compound, a preparation containing aloes as main ingredient, revealed that the drug was useful in cases of infertility associated with irregular menstrual cycles[39].

In another clinical study, Aloes Compound improved the fertility of patients with functional sterility and also improved menstrual functions in them. A sense of well being and improvement in health was observed in all patients on the drug. The

studies revealed that Aloes Compound is the drug of choice in cases of functional and disturbed menstrual function[40].

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Indian Aloe

V.K. Srivastava and B.M. Singh

National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi

ALOE is a genus belonging to the Liliaceae family. The word Aloe is said to be derived from Arabic word "Alloeh". Aloe genus comprises about 300 perennial species (Reynold, 1985). There are a number of species of Aloe introduced in India of which *Aloe barbadensis* Mill (Syn. *Aloe Vera* Linn) is almost naturalised in all parts of the country. Indian Aloe is known by different names in different parts of the country as shown below (Anon, 1985):

Hindi	-	Ghee Kanwar, Ghi-Kuvar
English	-	Aloes
Bengali & Sanskrit	-	Ghrita Kumari, Kanya
Gujarati	-	Kumarpathu, Kanawar
Kannada	-	Kolasoare, Komarika, Maulisara
Malayalam	-	Kottavazha
Marathi	-	Korphad
Tamil	-	Bhottu - katrazhae, Chirukattalai, Kottaalai
Telegu	-	Kalabanda
Oriya	-	Kumari, Mushaboro

Aloe is native to Africa, Canary islands, Spain and Mediterranean countries and has spread to East and West Indies, Southern USA, Central America, India, China and other countries. There are 2-3 easily recognisable varieties found in India. These are found in semi-wild state in all parts from the dry westward valleys of the Himalayas upto Cape Comarin. The plants are generally propagated by

suckers. *Aloe barbadensis* var. *chinensis* Baker is commonly found in Maharashtra, Karnataka, Tamil Nadu, Kerala, Andhra Pradesh and Madhya Pradesh. *Aloe barbadensis* var. *littoralis* Koenig ex Baker is found on the beach shingle in Tamil Nadu upto Rameshwaram. Another variety which thrives on the Gujarat and Bombay coast is the source of Jafrabad Aloe (*Aloe abyssinica* Baker).

The term 'aloe' stands for the dried juice which flows from the cuts transversely made over the large mature leaves of *Aloe* species. The aloe is known as 'Mussabar' in Indian market. For the preparation of aloe, the juice is allowed to ooze out from the cut leaves into a suitable vessel and then concentrated by evaporation either spontaneously or more frequently by boiling. The juice is colourless or yellowish to start with but darkens due to evaporation and boiling. The nature of the aloe depends upon the species from which it is prepared and the manner in which the juice is concentrated. If the juice is dried in the sun or concentrated over a low fire, it gives an amorphous opaque waxy extract called 'hepatic or livery aloe' due to resemblance of drug to liver tissues (Fassina, 1974). If the juice is concentrated rapidly over a strong fire, the material obtained on cooling is amorphous and semi-transparent. It is called 'glassy or viterous aloe'. Hence, all the aloes exist in both viterous and opaque modifications (Bhandari & Mukerji, 1959, Chopra *et al.* 1958).

The purgative activity of aloe is due to presence of anthraquinone compounds, aloin (Fairbairn, 1949). This was shown to occur in 85 out of 240 Aloe species (Reynold, 1985).

The three varieties of aloe drug are official in the Indian Pharmacopoeia:

(1) Curacao or Barbados aloe obtained from *Aloe barbadensis*;

(2) Socotrine aloe obtained from *Aloe perryi* and

(3) Cape aloe from *Aloe ferox* and its hybrids.

Besides above, aloe derived from *Aloe succotrina* is also in use.

Plant Description, Crop Production and Managment

A coarse looking perennial plant having short stem, shallow root system and found in semi-wild state in many parts of the country. It is about 2 feet high, bearing a rosette of large (about 15 inches long and four inches broad), thick (about 3/4 an inch) succulent, pale green convex leaves, tapers to a blunt point with horny prickles on the margin. Leaves are smooth and irregularly white blotched. Bright yellow coloured small tubular flowers are borne on a simple or branched scape originating from rosette. The flower is actinomorphic, its perianth is arranged in two whorls of 3 tapels each. It has six stamens in two whorls, the outer whorl has longer filament than the inner whorl. Ovary is superior, trilocular with axile placentation. The flowers usually appear during February and March which is also time for cutting

leaves. Leaves may be cut when one year old for collection of juice. Leaves left after collection of juice, are scrapped for mucilage which is used for preparation of Aloe gel. In this way leaves are cut for the extraction of juice and gel from the outer margin of the plants every year.

Cytological studies in *A. barbadensis* showed that the chromosome complement is bimodal and based on $2n = 14$ (Brandham, 1971). These include eight long and six short acrocentric chromosomes (Sapre, 1978). A triploid plant ($2n=21$) has been reported from monozite region of Kanya Kumari (Abraham and Prasad, 1979).

Examination of Aloe leaf sections reveals the presence of three type of cells at the phloem of the vascular bundles. These are aloin cells, outer bundle sheath cells and fibres (Cutler, 1980). The majority of the species have aloin cells of various sizes which produce a copius exudate containing the active principles of the aloe drug (Cutler, 1972). It was suggested that aloin cells act as storage tissue and those compounds are synthesised in the surrounding layer of cells usually one cell in thickness (Beaumount *et al.*, 1986).

Aloes are commonly cultivated for medicinal and cosmetic purposes. The most frequently employed species of the genus are *Aloe barbadensis*, *A. ferox*, *A. perryi* and a hybrid *A. ferox* × *africana* and *A. ferox* × *spicata* (Capasso and Donatelli, 1982). Thirteen out of 44 accessions collected within the country showed the absence of isobarbaloin compound. Obviously, these do not confirm to description of the taxa under *Aloe barbadensis* (Srivastava *et al.*, 1990).

In India, Aloe is cultivated on a limited scale in small scattered localities like Alwar in Rajasthan, Satnapalli in Andhra Pradesh and Rajpipla in Gujarat. This plant is usually vegetatively propagated due to widespread male sterility (Keizer and Cresti, 1987; Sapre, 1975). This plant flourishes in a variety of climates and even on the poorest of soils but needs protection from frost. *Aloe barbadensis* tolerates higher *p*H with high Na, K salts. For bigger plantation, suckers may be planted in rows 2 feet apart and 1 foot from plánt to plant spacing on a well prepared, manured loamy soil with plenty of broken bricks and coarse sand. It grows well in dry regions having low annual rainfall (5-30 cm) and at no time should soil become sodden (Verma, 1971). Leaves are harvested from outer margin of the plants every year. One acre field of Aloe produces about 15,000 to 35,000 kgs of fresh leaves annually. However, the yield of leaves declines after six years. It is also afflicted with leaf spot disease caused by *Alternaria alternata* and *Fusarium solani* (Roy, 1979).

Uses

Aloe has been used for therapeutic, cosmetics and miscellaneous purposes. The juice and pulp of leaves are usually used. The leaf exudate are used largely as a purgative and its parenchyma, against a range of skin lesions. Aloe drug is also

official in Indian Pharmacopoeia and British Pharmacopoeia. Aloe has many uses in folklore medicine (Karnick, 1972; Morton, 1961). In Ayurveda, it is described as follows (Satyavati *et al.*, 1976).

Bhedinee (purgation), netrya (beneficial to the eyes), rasayanee (rejuvenator), balya (muscular strength), vrishya (promotes virility), gulmaghña (indicated in tumor like conditions of the abdomen), vishagna (antidote for poison), pleeharo-gaghna (in splenomegaly), Yakritviddhihaara (cures hepatomegaly), Kaphajwara-hara (cures fevers caused by kapha), grantighna (cures glandular enlargement), agnidagdhashamanee (relieves the effect of burns), kushtaghna (in skin diseases), shwaasaghna (anti-spasmodic, anti-dyspnoic).

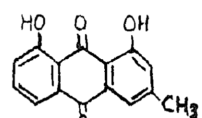
Aloe drug has wide range of therapeutic actions such as alterative, stomachic, cathartic, emmenogogic, astringent, antihelminthic, hepatic stimulant in spleen and liver ailments, stimulate hair growth, treatment of rectal fissures, X-ray and radiation burns, dermatitis, cutaneous leishmaniasis and other disorders of the skin (Cheney, 1970; Reynold, 1950; 1966; Yogi *et al.*, 1982; Row, 1940; Zawahry *et al.*, 1973; Blitz *et al.*, 1963; Hirata & Suga, 1977; Mckeown, 1983). It is also credited with antifertility or abortificient activity. The extract of the leaf inhibits the growth of micro-organism (Gottshall *et al.*, 1950). However, the purgative action of the aloe drug has received most detailed investigation. High biological activity of small doses of aloe drug is explained due to synergistic action of different aloe drug components (Fassina, 1974).

The second most important use of Aloe is in cosmetics in different blends. The mucilagenous pulp from the leaf parenchyma which is mainly carbohydrate in nature, is used in various types of skin disorders. A large number of research workers have published reports of their findings on the use of *Aloe vera* leaf in various skin conditions. Aloe-gel is the finer product of mucilage of leaf. It has ability to seal off the loss of moisture from the skin conditions. Aloe-gel has action on dead epithelial cells on the skin surface which causes ageing of skin due to cellular build up on the outer layer. Aloe-gel softens these dead cells and their removal from the surface leaving the skin smoother. Aloe-gel has an astringent action, besides, it has shown remarkable results on sun-damaged skins with its moisturing, softening and healing actions (Anon, 1987; 1977; Henry, 1979; Leung, 1977; 1978; Hofenberg, 1979).

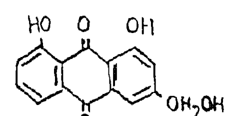
Besides, its use in medicine and cosmetics, the extract of *Aloe vera* is used for variety of purposes such as in the preparation of balms, different skin creams, suntan preparations, body lotions, shampoos, bath salts, soaps, talc, body powder, deter-gents and also used in soft drinks. In Gujarat, the leaves and flower stalks are pickled. The leaves yield a fibre, a dye prepared from this species gives purple colour to silk, black to wool and pink to linen. Jafarabad aloe is also used in lacquer work. In Kumaon, the leaf pulp is said to be eaten in times of extreme scarcity. (Watt, 1972; Chopra *et al.*, 1958).

Chemical Studies

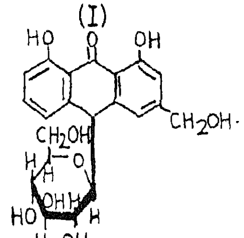
Leaf exudate of Aloe contains an array of chemical compounds. It contains aloins, a small amount of volatile oil, resin, gum, emodin, anthraquinone derivatives, chrysophanic acid and traces of coumarins (Chopra & Ghosh, 1938; Bhandari & Mukerji, 1959). Reynold (1985) carried out a detailed study of chemical compounds in Aloes. Because of their pharmaceutical activity, anthraquinones and their derivatives are the important one (Fairbairn, 1952; 1964). These occur either free (0.05-0.5%) or in the form of glycosides (5-25%) (Fairbairn,1949; Capasso & Donatelli, 1982). Free anthraquinone compounds are aloe-emodin (II) and chrysophanic acid (I) (Fassina, 1974). The purgative principle of Aloe has long been recognised as an anthracene glycoside (Fairbairn, 1949) and shown to be a C-glucoside of aloe-emodine anthrone (Birch & Danovan, 1955; Barnes & Holfeld, 1956). The compound was known as barbaloin (III) and characterised as 10 B-D-glucopyranosyl-1, 8-dihydroxy-3 hydroxy methyl-9 (10H) anthracenone (Hay & Haynes, 1956). A similar compound extracted from *A. barbadensis* has been characterised as 7-hydroxybarbaloin (Rauwald & Voetig,1982) and equated with isobarbaloin, This is probably a stereo-isomer of barbaloin or its artefact (Barnes & Holfeld,1956). Other major components of the drug aloe are two 5-mêthyl chromone-C-glycosides, aloesin (IV) or aloe resin B (Haynes et al.,1970), aloeresin A (Gramatica et al.,1982) and the aloesin aglycone, aloesone, (Holdsworth,1972).



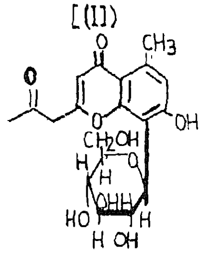
CHRYSOPHANOL
(I)



ALOE-EMODIN
[(II)



BARBALOIN
(III)



ALOESIN
(IV)

Fig. 1

The name aloin was given to a crude material from which barbaloin could be isolated (Lister & Pride, 1959), these two names have become interchangeable (Groom & Reynold,1987). The activity of barbaloin was ascribed to sugar group of the glycoside which allowed transport of the aglycone, i.e. anthraquinones intact into the site of action in the large intestine (Fairbairn,1964). This activity of the aglycone at the site of action depended on its state of oxidation and the presence and position of phenolic groups (Fairbairn, 1949). The level of barbaloin in commercial aloe product gives a more reliable measure of cathartic activity than the dosage of the crude drug (Mapp & McCarthy,1970). A great variability in barbaloin content, was observed within the genus, ranging from 2.5 to 30.8%. The content was also found to vary within the plant. There is more barbaloin in exudates of young mature leaves just below the apex and the level decreases in older leaves towards the base of the plant (Groom & Reynold,1987). The percentage of barbaloin in Indian Aloe populations ranged from 5.53 to 22.76% of dry weight of exudate The authors have shown that aloin content was positively correlated with short, broad and thick leaf types of Indian Aloe (Srivastava *et al.*,1990). It is shown that anthraquinones present in the plant were highest before flowering and then fell during growth process (Koshioka *et al.*,1982). The concentration of barbaloin in leaf exudate fluctuates during the year reaching a maximum during summer (McCarthy & Rheede van. Oudtshorn,1966), Climatic factors specially wind also influence the yield of barbaloin (Reynold, 1985). It is also shown that biosynthesis of aloenin take place during the spring (Suga *et al.*,1974).

The mucilage portion of the leaves contains glucose, galactose, mannose, galacturonic acid, an unidentified aldopentose, and protein with 18 amino acids (Elizabeth & Haagen Smith, 1948; Waller *et al.*, 1978). Aloe-gel a commercially important constituent of mucilage, is a polysaccharide with equal amount of glucose and mannose with small amounts of uronic acid (Shirolkar, 1987). In Indian Aloe populations it varies from 1.33 to 2.51 mg/ml of mucilage extract.

There are number of methods reported in the literature for the analysis of barbaloin content. The methods are based on Gravimetry (Lister & Pride 1959; Mahabale & Kamble, 1978), Thin Layer-Chromatography (Gerristsma *et al.*,1962; Boehm & Kreutzig, 1964), Colorimetry (Harder, 1949; Fairbairn & Simic, 1963; Krauss, 1959; Lemli, 1976), Gas-Liquid Chromatography (Takino & Ishii, 1978), High Pressure-Liquid Chromatography (Graaf & Alexa, 1980), Droplet Counter Current Chromatography (Rauwald, 1982) and Fluorophotometry (Ishii *et al.*, 1984).

Recently, number of active compounds were isolated from *A.barbadensis*. A compound aloe-feron was found to be highly bactericidal (Anon.,1985). Avocare Incorporation, Dallas, Texas, USA developed a compound named 'Carrisyn' from Aloe providing all the desirable properties attributed to Aloe. The studies have replication of the human cells. The increase in DNA synthesis preceedes an increase

in the rate of metabolic activity and cell replication, a fundamental step to healing process (Altman, 1985).

The biosynthetic pathways for anthraquinones were shown to involve either acetate and malonate or acetate, malonate and shikimate as starting material (Zenk & Leistner, 1968). The aloeemodine molecule appears to be built-up from acetate units through polyketide pathway (Simson 1980) and then converted to anthrone (Grün & Franz, 1982) before being combined with glucose to form C-glycoside (Grun & Franz, 1980; 1981; 1982). The acetate malonate route was shown for aloenin biosynthesis in Aloe acetate malonate route was shown for aloenin biosynthesis in Aloe (Hirata *et al.,* 1977; Suga & Hirata, 1978; Sugaa *et al.*,1974).

Conclusion and Prospects

Indian Aloe has a long history of its use in medicinal, cosmetic and many other purposes. The demand for this plant may likely to be increased due to staggering utilisation of natural medicinal products throughout the world. Hence there is an urgent need for increased production of this valuable material. *Aloe vera* is valued at about $ 30 to 40 million annually in global sale, (Anon., 1991). A market survey conducted in USA showed an eight fold increase in sales in the year 1982 as compared to 1981. In future, if the demand for Aloe and its commercial products increases, better planning of breeding startegy and identification of superior genotypes, will become a necessity.

Since biosynthesis of active principles are regulated genetically and large interspecific variability shown by the collections of Aloe, which can be utilised for cultivating desired type. Furthermore, there is a correlation of high aloin content in plant with its certain qualitative characters (Srivastava *et al.*, 1990). This can be exploited for producing better type. In this context, utilisation of *in vitro* culture has an important role to play such as clonal micropropagation method of *Aloe barbadensis* (Castorena Sanchez *et al.*, 1988). This may be used for rapid multiplication of favourable genotypes showing higher active principles with resistance to disease and climatic change. In spite of the importance of *in vitro* culture of Aloe species, it has yet to receive sufficient attention (Caavallini *et al.*,1991).

For better understanding of the accumulation of constituents of commercial importance in Aloe, there is also a need to study many secondary constituents, their biosynthetic pathway leading to their formation in plant and various factors affecting the biosynthetic pathway.

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Studies on Medico-ethnobotany, Diversity, Domestication and Utilization of *Picrorhiza kurroa* Royle ex Benth.

M.K.Kaul and Kiran Kaul (Thussu)

Regional Research Laboratory,
Jammu - 180 001, India

Introduction

P.KURROA is an important alpine herb of Himalayan region growing in an altitudinal range of 3000-5000 m in temperate belts. In Indian system of medicine it is known as "Kutki" and constitutes an important drug out of 2000 drug items derived from vegetable sources (Chopra, *et al.*, 1956). It is an Ayurvedic drug of great repute (Pandey, 1979). In Chinese system of medicine it is known as 'Hun-hung-lien'. However, it is known by different commercial and regional names in India. In Kashmir Himalaya, it is widely known as 'Kour'; in Himachal Himalaya it is readily identified by the name 'Kadu' and in UP Himalaya it is commonly called 'Kadvi'. The common English name is 'Hellebore', in Hindi it is called 'Katuka' whereas in Sanskrit it is known as 'Katurohini'. In higher reaches of the Himalaya it starts emerging with the melting of snow in summer and has a short life cycle of 4-5 months. The rootstock/ stoloniferous rhizomes running in the surface layers of soil are branched, rooting at nodes, up to 30 cm long and 0.3-1.0 cm in diam.

This drug has been thoroughly investigated both chemically and pharmacologically during the recent past but very little is known about its ethnobotanical, ecological and cultural aspects. Biological and chemical abstracts were screened to consolidate important chemical and pharmacological findings. In the recent years lot of emphasis is being laid on studying all aspects of herbal drugs to understand

these in modern scientific perspective and also reintroduce the confidence amongst the users. Among others Chandler *et al.*, (1979) and Fairbairn (1980) have stressed the need of authentication and standardization of herbal drugs. In view of the importance of this crude drug, huge quantities have been collected from the wild sources during the past and even at present it is being collected from its natural habitat. This indiscriminate extraction is bound to threaten its existence in the years to come. To save this plant from extinction and preserve its natural diversity and also assure the supply of quality raw material to phytopharmaceuticals, domestication studies at lower elevations (Fig. 1) were taken up by the author. The details regarding these studies are recorded in this paper.

Morphology and Drug Description

The herb is upto 20 cm tall at flowering stage in meadows of alpine region, possessing long branched stoloniferous rootstock which perennates during the most part of the year. The mature rootstock is covered allover with black remains of closely proximate scale leaves. Scars at different intervals on stolons, frequently in the form of protuberances, represent the accessory buds, roots and branches. The roots are seen to penetrate thin slaty or silty soils and at times anchored in rocky substrates. Stem is small, weak, creeping or erect at flowering, leafy and slightly hairy. Leaves mostly radical; cauline absent or appearing in the form of bracts at fruiting, spathulate and serrate, narrowing down into a winged petiole. Flowers very small in dense spicate racemes, bluish with exerted stamens which is characteristic of the inflorescence. Spikes 5-10 cm long, sub-cylindric, obtuse, many-flowered, sub-hirsute; bracts oblong or lanceolate, as along as the calyx. Capsule up to 1 cm long at maturity, with a long style dehiscing very late. The flowering occurs in mid summer (August). The sprouting of vegetative shoots starts with the melting of snow towards the end of June or early July. Drug of commerce is in the form of small pieces of rootstock (5 cm long and 1 cm diam.) collected in the month of October when the plants enter into dormancy. These are generally greyish or dark brown with rough texture and small scars.

Ethnobotanical Observations

Ethnobotanical studies carried out by the author in North-West Himalayan regions during the last decade have shown that this drug is holding maximum repute in view of its efficacy to alleviate human sufferings. "Sir J D Hooker while in Upper Sikkim in late nineteenth century received from salt traders a handful of roots of one of the many bitter herbs called Bengal teeta (*Coptis teeta*), but he adds that the present was that of *Picrorhiza kurroa*, a powerful bitter herb" (from Watt, 1892). There is hardly any member of hill tribes living in higher Himalaya who is ignorant about the properties of this herb. The ethnobotanical information given in Table I is mostly from author's personal records. However, some information has been

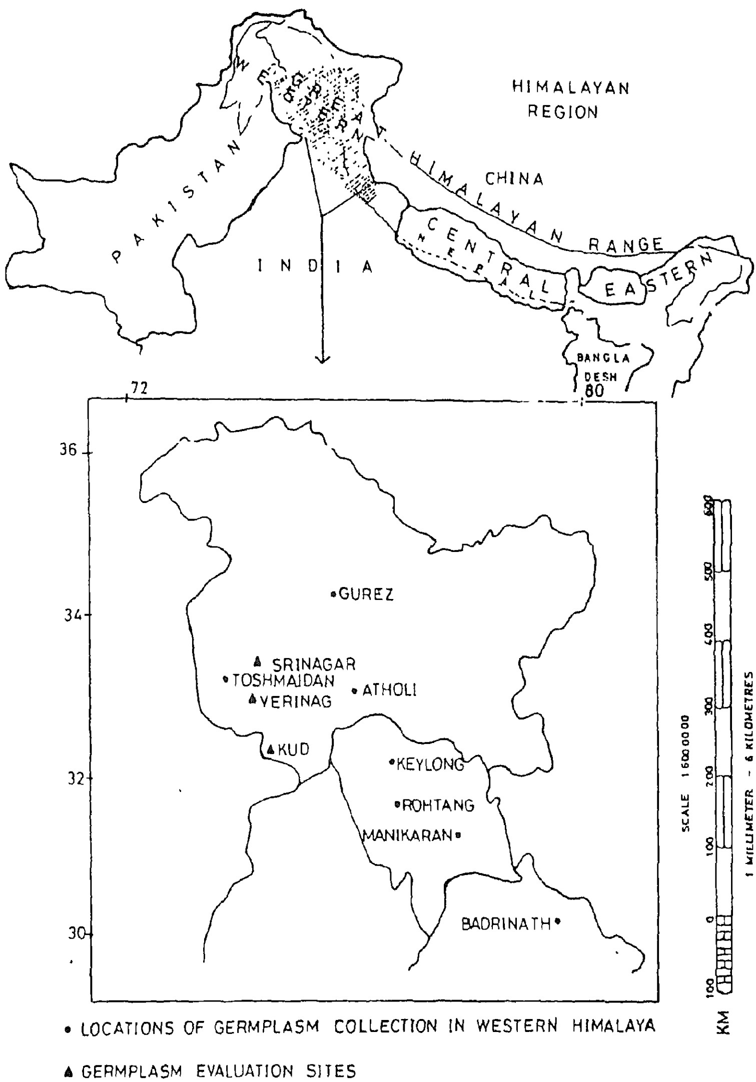

Fig 1 — Germplasm Collection Sites and evaluation Centres

recorded from literature. Nomadic communities from the Himalaya keep this drug at their places for use as a home remedy for various gastrointestinal disorders. Bhattarai (1992) from Nepal Himalaya has reported that the root paste is applied to cuts and wounds for speedy healing. It is also said to be antiseptic. Apart from this it is used as a ethnoveterinary medicine. The decoction of root in water is given with salt to fabrile cattle as antipyretic.

Table 1 — Ethno-botanical notes on *P.kurroa* in Himalaya

Region/ Locality	Local name	Frequency of occurrence	Traditional use
I.*Kashmir Himalaya*			
Aporwat	Kode	Rare	Bitter tonic given in liver disorders
Gulmarg	Kode	Rare	Bitter tonic
Gurez	Kour	Common	Stomachic, mild purgative
Kangan	Kode	Occasional	Against stomach ache and dyspepsia
Karna	Kadu	Occasional	Against dyspepsia
Keran	Kour	Rare	Bitter tonic, given against mild fevers
Lolab	Kadu	Rare	Mild purgative
Maitchel	Kour	Occasional	Stomachic, remedy for mild fevers
Padar	Kour	Occasional	Respiratory disorders, given in jaundice
Tilail	Kour	Common	Stomachic, mild purgative
II.*Himachal Himalaya*			
Chamba	Kadu	Common	Bitter tonic, antiperiodic
Keylong	Kadu	Occasional	Respiratory problems and dyspepsia
Manikaran	Kadu	Rare	Mild laxative, dyspepsia and liver complaints
Pangi	Kutki	Common	Bitter tonic, antiperiodic
Rohtang	Kuru	Occasional	Liver disorders and against asthma
III.*UP Himalaya*			
Badrinath	Kadvi	Occasional	Against liver disorders and dyspepsia
Chamoli	Karu	Rare	Bilious fevers, bitter tonic
Gopeshwar	Kadvi	Rare	Bilious fevers, bitter tonic
Kidarnath	Kadvi	Occasional	Respiratory problems
Uttar Kashi	Kuru	Occasional	Jaundice remedy
IV.*Nepal Himalaya*			
Kathmandu	Kutki	Occasional	Rootpaste antiseptic; root decoction antipyretic
V. *Eastern Himalaya*			
Sikkim	Kutki	Occasional	Bitter tonic, mild laxative

Traditional and Modern Use

This herb was known to be wonder drug before 1000 B.C. In Sanskrit the root is described as bitter, acrid and stomachic in smaller doses and moderate cathartic in larger doses. It was considered to be highly useful in fever and dyspepsia and an ingredient of various purgative combinations. Frequent mention has been made about this plant in Ayurveda which is considered to be the oldest system of Indian medicine. Dymock (1891) in his Pharmacographica Indica writes" I can state from my personal observations that it is used successfully as an antiperiodic in native practice in Bombay; its slight laxative action is rather beneficial than otherwise". Dose : as a tonic 10-20 grains; as an antiperiodic 40-50 grains. Administered in combination with aromatics in view of its bitter taste. As per Dymock two forms of the drug were sold in Bengal, India under the name "Kuru" and "Katki"- sometimes roots of *Gentiana kurroa* and that of *Coscinium fenestratum*. Gimlett, Residency Surgeon in Nepal states that it is brought from Tibetian border and the root is used in the enlargement of spleen (vide Watt, 1892). The drug is a major component of "Agrogyavardhini" - a potent Ayurvedic formulation used to cure liver disorders. The traditional medicinemen have been using this drug to cure cirrhosis of liver (Bhagwan Dash, 1979).

Recently the drug has been reported to be hepatoprotective and a bile flow enhancer (Handa *et al.*, 1986). Antiasthmatic effects have been observed in patients with chronic asthma. Immunomodulating activity has been recently reported (Atal *et al.*, 1986). Immuno-potentiating efficacy of this drug as a supplement to photochemotherapy in patients with vitilago has been studied by Bedi *et al.*, (1989).

Adulterants

In view of demand of this drug in the market, from centuries some other bitter plants are being sold under the name 'Kutki'. Dymock (1981) observed that roots/root chips of *Actaea spicata, Cimcifuga foetida* and *Coptis teeta* were sold in drug exhibitions at Bombay under the name 'Kuru' or 'Kutki'. Similarly at Bengal drug shops the roots of *Gentiana kurroa, Coscinium fenestratum* and *Swertia chirata* were offered as 'Kuru'. Recently Dhawan *et, al.*, (1991) have reported *Lagotis cashmiriana* as an adulterant of commercial samples of *P. kurroa*. It is reported that at times *L. cashmiriana* is sold in the name of 'Kutki' in Kulu market of Himachal Pradesh. The morphological similarities have been described. However, absence of hepatoprotective activity has been recorded in roots of *L. cashmiriana* (Dhawan *et al.*, 1991). The author procured drug samples of Kutki from established drug markets of Baramulla (Kashmir), Kulu (Himachal Pradesh), Tanakpur (Uttar Pradesh), Amritsar (Punjab), Bombay (Maharasthra) and Calcutta (W. Bengal) for authentication and chemical evaluation. It was observed that the samples sold in the drug markets in India are by and large genuine. However, qualitywise there was

lot of difference which can be attributed to collection time, age of the plant and region of collection. The kutkin content in market samples showed a big range of (0.5 - 3.5 %, Table. 6).

Distribution, Availability and Ecological Status in Himalaya

The botanical survey undertaken by the author in the Himalayan region of three states viz., Jammu and Kashmir, Himachal Pradesh, and Uttar Pradesh revealed that *P. kurroa* grows luxuriantly on open exposed slopes of higher plateaus above tree line. It is distributed all over Himalaya except the high rain-fed belt of Eastern Himalaya (Fig.2). In Kashmir Himalaya it grows in high reaches of Gurez valley,

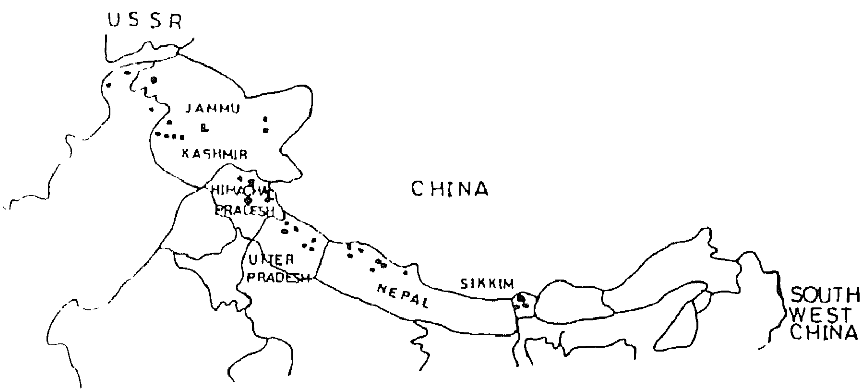

Fig 2 — Distribution of *Picrorhiza kurroa* in the Himalaya

Lolab, Karna, Sindh and Lidder valleys. It is commonly seen associated with the herbs like: *Aconitum violaceum, Lagotis cashmiriana, Potentilla kashmirica, Sedum ewersii* and *Senecio jacquemontiana*. It is observed to be common in Gurez, occasional in Lolab and karna valleys, whereas rare in Sindh valley (Tab. 2). On the whole from Kashmir Himalaya this species is considered to be endangered one (Table. 2). As per reports the herb has been extracted for centuries from this region. During the years 1925-1927, about 11 tonnes dry roots were extracted annually as per estimates of the Forest Department, J&K (Kaul, 1928). In 1985 it was estimated to be 5 tonnes. Minor Forest associations recorded are : *Agrimonia eupatoria, Geum urbanum, Polygonum* spp., *Potentilla sibaldii* and *Thalictrum minus*. It is reported to be common in Chamba and Pangi valleys and occasional in Lahul valley, whereas rare in Parvati valley. On this whole in Himachal Pradesh the species is vulnerable (Table 2). There are no figures of extraction available. However, annual availability

Table 2 — Representation of *P. kurroa* in Himalaya

Himalayan range	Ecological status	Genetic diversity	Economic significance
Kashmir	E	VH	VH
Himachal Himalaya	V	H	VH
Garhwal Himalaya	E	VH	VH
Central Himalaya	NK	VH	VH
Eastern Himalaya	V	H	VH

Legend: E - Endangered; V - Vulnerable; NK - Not known; VH - Vary high; H - High

from 23 forest divisions of Himachal Pradesh is reported to be 3 tonnes (Sarin and Chopra, 1985). From UP Himalaya it is reported to be an endangered species (Table 2). It is being extracted indiscriminately from Badrinath, Kidarnath and Chamba areas. In Garhwal Himalaya the most common associations recorded are: *Gaultheria trichophylla, Potentilla sibaldii* and *Rhododendron anthopogon*. From Nepal and Sikkim Himalaya no figures are available about its extraction. However, lot of material is exported from this region to other states and the drug collected from this region is available in crude drug markets of India.

As per author's personal observations on the ecological status of the herb, it is concluded beyond any doubt that the plant will not survive any more rigorous extraction and the species will be rare by the end of the century. Steps towards its conservation are needed to protect its genetic stock in the Himalaya.

Diversity and Domestication

The antiquity of the Himalaya as a seat for diversity of medicinal plants is well-known and actual diversity of most of these plants is unknown. Although *P. kurroa* has been extracted from earlier times, yet its genetic base is relatively rapidly eroded during the past two decades. This is because, during the recent past higher reaches in Himalaya have been exposed to more biotic interference. In view of endangered ecological status of this species, a strong need was felt to domesticate it at lower elevations and study its cultural behaviour. There is no record of its organised cultivation.

During the year 1988, a modest beginning was made to study the acclimatization of this high altitude alpine herb at lower elevations. The domestication trials were laid at three different locations having different agro-climatic status. The locations are : 1) Experimental plots of Regional Research Laboratory, Srinagar (1600 m); 2) Campus of High Altitude Research Laboratory, Gulmarg (2700 m); and 3) Experimental station of RRL at Verinag, Kashmir (3050 m). In the years 1990-1992, the domestication trials were laid at Forest Experimental Station, Kud, Jammu (1750 m).

Germplasm Evaluation at Srinagar, Gulmarg and Verinag

Fresh germplasm was collected from higher reaches of Toshmaidan and Gurez valley in Kashmir Himalaya during the months of August and Sepetember, 1988. The morphological variability recorded from the germplasm procured showed that the length of rhizomatous stolons ranged from 12.5 - 14.4 cm with slight variation in colour; diameter ranged from 0.3-0.6 cm and leaf length, shape and texture was more or less similar with minor differences (Table 3). As regards spike length, it ranged from 4.5-6.0 cm. Chemical analysis showed that average Kutkin content of rhizomes procured from Padar, Toshmaidan and Gurez was 1.80%, 1.08% and 0.840% respectively (Table 5). Random rhizome and leaf samples were sent to Central Drug Research Institute, Lucknow, India for checking hepatoprotective activity in rats. The data recorded in Table 4 shows that rhizome samples of Gurez germplasm showed 60- 90% protection in galactosamine model, whereas those of Toshmaidan germplasm showed 40-45% protection. No activity was recorded in leaf samples.

Table 3 — Morphological variability in germplasm procured from North-west Himalaya (Data taken as mean of one hundred plants)

Plant part/Trait		Germplasm Source						
		Kashmir Himlaya			Himachal Himalaya		UP Himalaya	
		Gurez	Tosh	Padar	Roht.	Keyl.	Manik.	Badrinath
a)	Rootstock/ Rhizomatous stolon							
i.	Length/plant (cm)	14.4	18.3	12.5	11.5	10.9	12.3	19.5
ii	Diam. (cm)	0.3	0.4	0.6	0.4	0.5	0.8	1.2
iii.	Colour	Greyish balck	Greyish	Pale brown	Grey brown	Grey brown	Blackish brown	Blackish
b)	Leaf							
i.	LxB (cm)	5.5×2.1	6.2×3	7×3.1	5.1×3.9	6×4.2	6.5×4.5	7.5×4.2
ii.	Texture	Smooth	Smooth	Slight hairy	Hairy	Smooth	Hairy	Hairy
iii.	Shape	Obovate	Ovate	Oblance -eolate	Ovate	Obovate	Obov.	Oblanceolate
c)	Inflorescence							
i.	Peduncle length(cm)	6.5	8.0	5.5	7.0	8 5	8.2	9.5
ii.	Spike length(cm)	4.5	5.0	6.5	6.2	7.6	7 1	8.7
iii.	Texture	Slight Hairy	Slight Hairy	Hairy	Hairy	Slight Hairy	Smooth	Hairy

Table 4 — Pharmacological activity of germplasm procured from Kashmir Himalaya*

Germplasm source	Part	Date of collection	Pharmacological activity
Gurez (Kashmir)	Rhizome	July,1989	60-90% protection in galactosamine model
Gurez (Kashmir)	Leaves	July,1989	No activity
Toshmaidan (Kashmir)	Rhizome	August,1989	40-45% protection in galactosamine model

*Courtesy . CDRI Lucknow, India

Mostly crown splits and growing buds separated out from the wild germplasm planted in the experimental plots at Srinagar, Gulmarg and Verinag in the month of September, 1988 remained under snow during winter months. The plantation was done in ridges with row to row distance of 30 cm and plant to plant distance of 10 cm. Sprouting was observed from ending March, 89 to ending April at different locations with Srinagar plants coming out first. Under Srinagar conditions 70% plants showed initial survival, but with the day by day rise in temperature the plants showed senescence Artificial shade was provided and the plants started looking healthy and in the population 60% survival was observed. Only 8% plants flowered at the end of May, 89 and thrived very well in open conditions as well as shade of coniferous trees - *Cedrus deodara* and *Abies webbiana*. About 80% plants survived and 20% flowering was observed in the population. In Verinag experimental plots the plants sprouted towards the end of April, 89 and 80% survival was recorded. No flowering was observed as the plants could not survive high temperatures of May,89 (average 30°C).

Germplasm Evaluation at Kud, Jammu Hills

Fresh germplasm collected from Himachal and Garhwal Himalaya during the months of August, 90 and July, 91 was brought in live condition for plantation at Forest Experimental Station, Kud in Jammu hills (Fig. 1). The morphological variability recorded showed that the length of rhizomatous stolons ranged from 10.9 - 12.3 cm and diameter from 0.4 - 0.8 cm in the plants collected from different regions of Himachal Himalaya. The plants collected from Garhwal Himalaya showed an average length of 19.5 cm and diam 1.2 cm (Tab. 3). The colour of rhizomes ranged from greyish brown to blackish brown. Leaf length, size and texture was more or less similar. However, the inflorescence was hairy to smooth in Himachal Plants and hairy in Garhwal plants. Kutkin content of rhizomes varied from 0.7 - 0.9% in Himachal plants, whereas it was 1.0% in Garhwal ones, (Table 5) Crown splits with 5 cm long rhizomes were separated out and planted in ridges

with the same planting distance as kept under Srinagar conditions. The plantation was done in August, 90 and July, 91 immediately after procuring live germplasm from higher reaches in Himachal Himalaya and Garhwal Himalaya. The plants remained under snow for not more than 40 days and sprouted in early March of the subsequent year of plantation. Only 25% plants survived in exposed situations, whereas 60% plants survived in shady conditions. The plants did not resist temperatures beyond 30°C. No flowering was observed under Jammu hill conditions.

Table 5 — Kutkin content of germplasm procured from different geographical regions

Germplasm source	Plant part	Date of collection	Percentage Kutkin
Gurez	Rhizome	July,1989	0.840
(Kashmir)	Rootlet	-do-	0.510
Toshmaidan	Rhizome	August,1989	1.080
(Kashmir)	Rootlet	-do-	0.710
Padar	Rhizome	July,1992	1.80
(Kashmir)	Rootlet	-do-	0.470
Keylong	Rhizome	June,1991	0 70
(Himachal)	Rootlet	-do-	0.40
Rohtang (Himachal)	Rhizome	June,1991	0.90
Garhwal	Rhizome	Aug.,1991	1.0
(U.P)	Rootlet	-do-	0 70

Seed germination

Under natural conditions seeds get dehisced during the months of Autumn and remain under snow for six to seven months germinating readily with the melting of snow and forming small colonies of seedlings. Freshly collected seeds did not germinate profusely under laboratory conditions. Chilling at 4°C for 1-3 months was necessary for stimulating germination. Maximum germination of 55% was observed in seeds chilled for 3 months. This behaviour confirms the alpine character of the herb. Chilled seeds germinate readily at 20°C. The seedlings obtained under laboratory conditions showed high mortality when transferred to normal garden soil, but the seedling survival improved highly when the soil from natural habitats was used It seems there in some inhibiting factor in normal garden soil

Clonal Propagation

Fresh rhizomatous stolons collected from natural sites during autumn - just before entering dormant phase were kept at 4°C under laboratory conditions. The growing buds all along the stolon length started sprouting (Fig. 5). Stolon cuttings with

growing buds were planted in pots as well as in field at Kud experimental station. Initially 80% survival was seen but subsequently high mortality rate was observed. Stolon splits were treated with different hormones like Indole-acetic acid and Indole-butyric acid and planted in pots as well as field conditions. In pot culture IBA showed encouraging results (60% stolon splits rooted). It is seen that under 'ex situ' conditions clonal propagation is a better method of propagation (Fig. 4).

Kutkin Content in Domesticated Stock

The samples of rootstock/rhizomes were drawn from one year and two year old plantations from domesticated germplasm stocks at RRL (Br) Srinagar experimental plots in the years 1989 and 1990. During the years 1991 and 1992, samples were drawn from one year old plantation at Kud, Jammu hills. These samples were analysed for Kutkin content in the Chemistry Division of RRL, Jammu. From the chemical analyses it is seen that the variability exists in Kutkin content of different germplasm stocks (Tab. 7). It is observed that the kutkin content improves with the advancement of age. The main rootstock has, on an average, double the content of active principle (kutkin) as compared to rootlets.

Chemical Composition

The main glycoside reported from the rhizomes/rootstock of *P. kurroa* is Kutkin. It is a crystalline compound (earlier believed to be a single compound), reported to be a mixture of two C-9- iridoid compounds - Picroside I (6'-o-trans-cinnamoyl catalpol) and Kutkoside (10-o-Vanilloyl Catalpol). A novel curcurbitacin glycoside (25-acetoxy-2- glucosyloxy-3,16,20-trihydroxy-9-methyl- 19-norlanosta-5, 23-diene-22-one) was found to be more bitter than Picroside I and II (vide *Chem. Abstr.* 1986, 104, 48708e). From the roots an iridoid glycoside (6-feruloylcatalpol) alongwith Vernicoside and minecoside and two phenol glycosides, picein and androsin were obtained (Stupner and Wagner, 1989). From the aqueous sodium carbonate extract of *P. kurroa* ten compounds were isolated as crystalline acetates (*Chem. Abstr.* 1990, 112, 4544J). Seven new cucurbitacins were isolated from roots of *P. Kurroa* (Stuppner, Miller and Wagner, 1991).

Table 6 — Kutkin content of market samples collected/procured from different drug markets in India

Market source	Part/stock year	Percentage kutkin
Amritsar	Rhizome/1990	1.5-3.5
Bandipur	Rhizome/1989	0.5-1.5
Bombay	Rhizome/1990	2.0
Baramulla	Rhizome/1989	0.5-1.5
Calcutta	Rhizome/1990	2.1
Tanakpur	Rhizome/1991	2.0-3.0

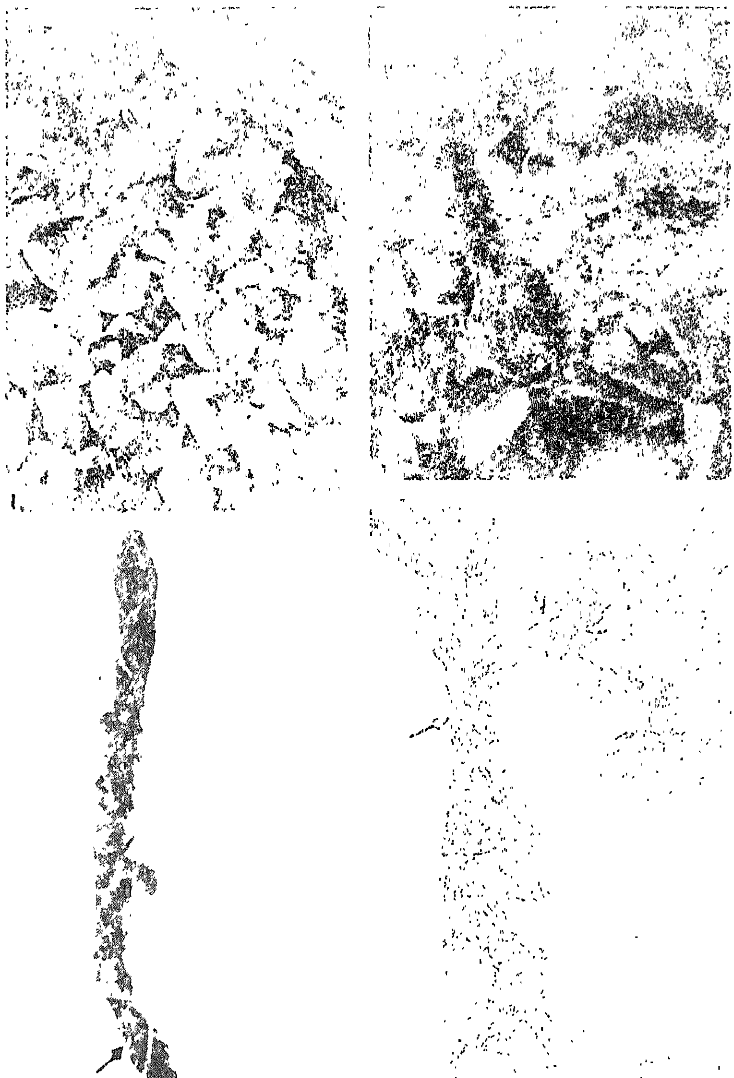

Fig. 3 — *P. kurroa*, A plant in flowering (1) *Picrorhiza kurroa* a wild population, (2) A plant in flowering, (3) Rhizomatous stolon showing growing buds in spring, (4) Rhizome crown showing roots and growing buds.

Table 7 — Kutkin content of domesticated stock

Stock	Plant part	Age	Percentage kutkin
Srinagar Trial (1988-89)			
PKT	Rhizome	1 year	0.85
	Rootlet	1 year	0.33
PKG	Rhizome	1 year	0.88
	Rootlet	1 year	0.41
Gulmarg Trial (1988-89)			
PKT	Rhizome	1 year	1.91
	Rootlet	1 year	0.90
Kud Trial			
PKR	Rhizome	1 year	0.90
	Rootlet	1 year	0.51
	Rhizome	2 year	1.50
	Rootlet	2 year	0.80
PKP	Rhizome	1 year	1.25
	Rootlet	1 year	0.71
	Rhizome	2 year	1.90
	Rootlet	2 year	0.61

Legend: PKT - Germplasm from Toshmaidan,Kashmir
PKG - Germplasm from Gurez, Kashmir
PKP - Germplasm from Padar, Kashmir
PKR - Germplasm from Rohtang, Himachal

Pharmacological Studies

Picroside II and related glycosides have protective effect against carbon tetrachloride induced liver intoxication (*Chem. Abstr.* 1973, 79, 108054r). It also rendered guinea pigs less sensitive to histamine (*Chem Abstr.* 1977, 86, 101035w). It is reported to possess immunomodulating properties (Atal *et al.*, 1986). The iridoid mixture from rhizomes of *P. kurroa* exhibited hepatoprotective activity against liver damage induced by galactosamine in rats (*Chem. Abstr.* 1988, 786e). It is reported to potentiate photochemotherapy in vitilago which could be due to summation of its immunomodulating and hepatoprotective actions (Bed *et al.*, 1989). Protection against *Amanita phalloides* poisoning in mice by kutkin isolated from rhziomes is reported. The protective effect was comparable to that of Silibinin; the curative effect of kutkin being superior (*Chem. Abstr.* 1990, 112, 93585, 93588s). It is reported to prevent Paracetmol-induced hepatic damage in rats (*Chem. Abstr.* 1991, 115, 85378, 85375s). Picroliv is reported to be hepatoprotec-

tive in rats (Dhawan *et al.*, 1991). It is reported to reverse cholestasis induced by thio-acetamide in rats. Immunostimulent activity of Picroliv and also protective action against *Leishmania donovani* infection is reported by Puri *et al.*, 1992).

Results and Discussion

Picrorhiza kurroa is undoubtedly an important medicinal herb of traditional and modern systems of medicine. Apart from its use as stomachic, aperient, antiperiodic and cholagogue in traditional medicine, it is at present reported to be used as hepatoprotective, immunomodulator, antiasthamatic and immuno-potentiating agent to supplement photochemotherapy in patients with vitilago (Atal *et al.*, 1986; Handa *et al.*, 1986; Bedi *et al.*, 1989). Ethno-medicobotanical studies have shown that this herb has wider utilization in the Himalayan region (Tab. 1). In addition to its use against human diseases, it is reported to have ethnoveterinary significance in Nepal Himalaya (Bhattarai, 1992).

The present studies have shown that Himalayan plants show lot of variability-morphologically as well as chemically. Qualitatively the genetic stock of central and Eastern Himalaya is better than that of north-west Himalaya. The rhizomatous stolons are longer, thicker and unbranched in the plants of Garhwal and Sikkim which is supposed to be a superior trait. The superiority of this stock is confirmed chemically too.

Many herbs have been used conventionally as adulterants to this herb in the Himalaya. Crude drug markets in India and China are dependent on supply from wild sources. Indiscriminate collection from natural habitat is bound to threaten its survival by the end of this century. Present studies have confirmed its disturbed ecological status. Systematic efforts initiated to domesticate this species at lower elevations have shown encouraging results. The herb has been successfully domesticated at Srinagar, Kashmir. The plants responded well to the treatment of growth hormones for inducing rooting. Germination is poor under 'ex situ' conditions and vegetative multiplications shows encouraging results. Chemical evaluation of domesticated stock showed that there was no significant change in kutkin content in first two years of growth. On an average the main rhizome contains double kutkin content than the finer roots and branches. There is lot of variation in kutkin content of the plants collected from different regions. Market samples were seen to possess more kutkin content than the fresh germplasm collected during the months of July and August. This can be attributed to the stage of harvest and the age of the plants. Normally organised collection of the drug is done in the month of October (post fruiting stage, when the plants start entering dormancy). It seems kutkin content is optimum at this stage. In fact, further studies are needed to ascertain the role of age and stage of harvest on kutkin content.

Conclusion

From the above studies it is concluded that:

1. *Picrorhiza kurroa* is a drug of future in view of its hepatoprotective and immunomodulating properties. More chemical and pharmacological studies are needed to unfold the mysterious properties of this herb.
2. Its extraction from wild sources should be banned in view of its present ecological status.
3. Organised cultivation should be undertaken at lower elevations to provide quality raw material to the user industry.
4. The plant exhibits lot of phenotypic and chemotypic variation in nature. This variability can be exploited to produce better, high kutkin yielding strains.
5. The active principle - kutkin is optimum at post-fruiting stage when the plants start entering dormancy.
6. Clonal propagation through vegetative multiplication and involvement of tissue culture techniques should be encouraged.

Acknowledgements

The author places on record the timely help and encouragement of the following scientific and administrative persons who have contributed towards the completion of this work:

Dr.R.S.Kapil, Director, Regional Research Laboratory, Jammu was kind enough to provide various institutional facilities. Dr.P.K.Sharma, Dr.V.Singh, Mr.S.M.Shafi, Mr.Ahmad Malik accompanied the senior author to various inaccessible regions in the higher Himalaya to procure the live germplasm for further studies. The authorities of Forest Department, Jammu and Kashmir and Himachal Pradesh were kind enough to provide facilities for field experimentation. The work could not have reached to a meaningful conclusion without the timely chemical back-up given by Dr.O.P.Suri and his group working in Chemistry Division of RRL, Jammu.

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Iridoids and Secoiridoids of the Genus *Swertia*

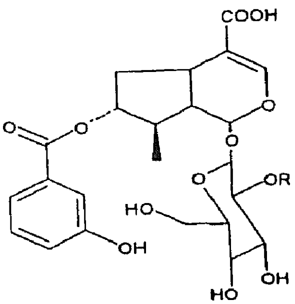
Maninder, Karan Vasisht* and S.S. Handa

Regional Research Laboratory, Canal Road, Jammu Tawi

THE iridoids and secoiridoids constitute the bitter principles of the genus *Swertia*. Among the iridoids, three compounds are reported from a Japanese species, *S. japonica,* which are swertiaside [7-*epi*-(*m*-hydroxybenzoyl)-loganic acid] (1),[1] senburiside I [7-*epi*-(*m*-hydroxybenzoyl)-2′-sinapoyl loganic acid] (2)[2] and senburiside II [7-*epi*-(di-*m*-hydroxybenzoyl)-loganic acid](3)[3]. Senburiside I contains a novel sinapoyl moiety linked to the glucose residue in the molecule. The presence of these 7- *epi*-loganic acid derivatives in this plant warrants interest from biogenetic and chemotaxonomic stand points, since, hitherto, only loganin and loganic acid derivatives were known from the genus.

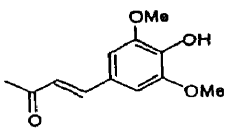
A number of secoiridoids namely swertiamarin (4), 2′-*O*-acetylswertiamarin (5), sweroside(6), gentiopicroside(7), amarogentin(8), amaroswerin(9), amarogentin B (10), swertiapunimarin (11), augustiamarin (12), augustioside(13), epieustomoside, nervoside and vegeloside have been isolated from a number of *Swertia* species. The group is led by swertiamarin (4) which was the first to be isolated from *S. japonica* by Kariyone and Matsushima in 1927. It's chemical composition was determined a year later in 1928[4]. Enzymatic hydrolysis of swertiamarin with emulsin affords erythrocentaurin and glucose[5]. Swertiamarin is also the most commonly distributed bitter in the genus *Swertia* reported from *S. alata,*[6] *S. angustifolia,*[7] *S. augustifolia,*[8] *S. chirata,*[9] *S. mileensis,*[10] *S. nervosa,*[11] *S. patens,*[12]

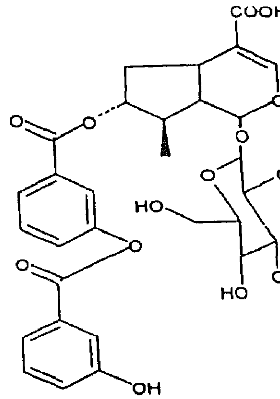
*University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh



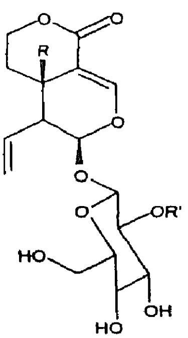
(1) R=H

(2) R=





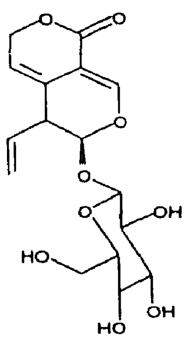
(3)



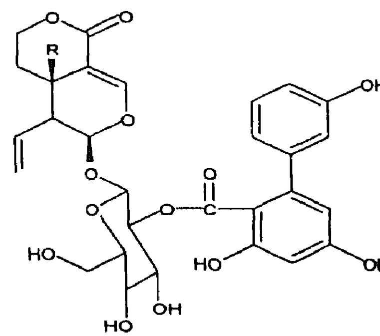
(4) R=OH, R'=H

(5) R=OH, R'=Ac

(6) R=H, R'=H

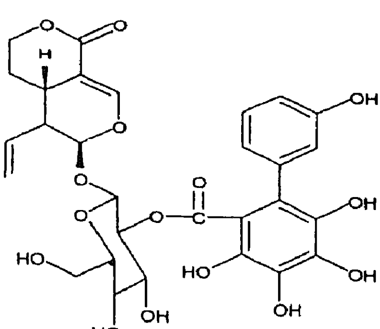


(7)



(8) R=H

(9) R=OH



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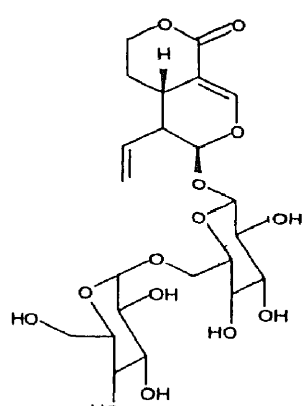

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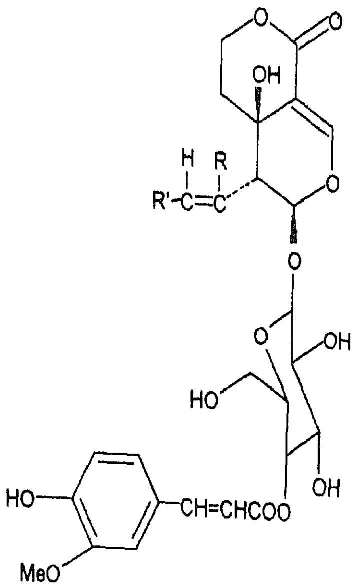
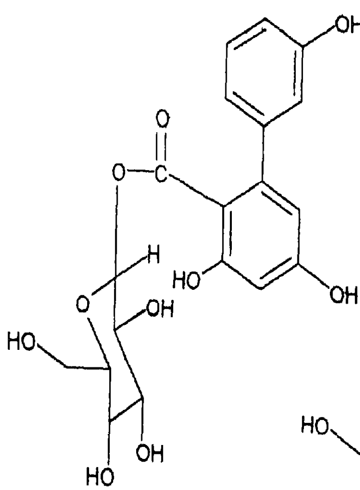


(12) R=R'=H

(13) R,R'=O



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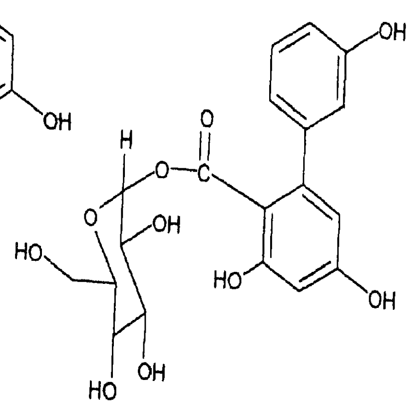

(15)

S. punicea,[13] *S. purpurascens,*[14] *S. tetrapetala,*[15] *S. pseudochinensis*[16] and *S. japonica*[17-19]. 2′-*O*-Acetylswertiamarin(5), which carries monoacetylated glucose residue, is reported from *S. mileensis*[20]. Sweroside (6) is structurally related to swertiamarin, the latter being 5-hydroxy derivative of sweroside. It is involved in a number of chemical studies carried to investigate the biogenetic relationship of secoiridoid glucosides. The labelled DL- mevalonolactone-2-^{14}C is metabolized to sweroside, swertiamarin and gentiopicroside by *S. japonica* which suggest the biogenetic origin of gentiopicroside from sweroside via swertiamarin. The pathway involves hydroxylation of sweroside to yield swertiamarin which on dehydration results into gentiopicroside[21-23]. Sweroside is reported from a number of *Swertia* species viz. *S. arisanensis,*[24] *S. augustifolia,*[8] *S. japonica,*[17-25] *S. nervosa*[11] and *S. punicea*[13]. Gentiopicroside (7) (gentiopicrin of the old nomenclature) is the earliest known bitter constituent of Gentianaceous plants, whose isolation from *Gentiana lutea* dates back to 1862 though its chemical structure was identified 100 years later in 1961[26-28]. In the genus *Swertia,* it was initially isolated from *S. chirata*[29] in 1955 and thereafter reported from *S. caroliniensis,*[30] *S. japonica*[17] and recently from *S. punicea*[13]. Gentiopicroside is water soluble having bitter value 12,000. In presence of ammonia it easily degrades and yields gentianine[31].

Amarogentin(8), isolated from *S. chirata* in 1955, is the bitterest plant product known till date. It's bitter taste is detectable even at a dilution of 1 part per 60 million and is 10 times bitter than brucine[29,32]. The structure of amarogentin has been established as sweroside-2′-3″,5″,3‴-trihydroxydiphenyl-2″-carboxylic acid ester[33,34]. Amaroswerin (9) (swertiamarin-2′-3″,5″,3‴-trihydroxydiphenyl-2″-carboxylic acid ester) is another intensely bitter secoiridoid glucoside structurally related to amarogentin being its 5-hydroxy derivative[33,34]. The biphenyl carboxylic acid moiety of these two strongly bitter secoiridoids (8, 9) is biosynthesized from three acetate units and a 3-hydroxybenzoic acid, the latter coming from phenylalanine via benzoic acid[35]. This moiety itself being bitter as indicated by its glycosyl esters, biphenoside A & B (14, 15) adds to the bitter taste of secoiridoid segment which explains the extremely bitter nature of amarogentin and amaroswerin[36]. Amarogentin and amaroswerin are also reported from *S. japonica*[2,33,36] and *S. punicea*[16]. Amarogentin B (10) reported from *S. punicea* also carries a biphenyl carboxylic acid moiety which is in more oxygenated state[16]. From the same plant, swertiapunimarin (11) has recently been reported which is a disaccharide glycoside[37]. The three new secoiridoid glycosides augustiamarin, augustioside and epieustomoside have recently been isolated and identified from *S. augustifolia*[8]. Augustiamarin (12) and augustioside (13) carry the aglycone structure of swertiamarin. Two new secoiridoid glycosides nervoside and vegeloside have been reported from *S. nervosa*[11].

Several studies on the analysis of the secoiridoid glycosides for the chemical evaluation of the *Swertia* species have been carried out which include rotation locular counter current chromatography, thin layer chromatographic (TLC) analy-

sis of swertiamarin and high performance liquid chromatographic (HPLC) analysis for swertiamarin, gentiopicroside, amaroswerin and amarogentin[38-42]. However, a serial analysis and systematic separation of secoiridoids was achieved by solvent partition and HPLC and the method was applied for the quantitative analysis of the glycosides in *S. japonica* and *S.pseudochinensis*[43].

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Technology of Podophyllotoxins

S.M.Anand, S M Jain and R S.Kapil

Regional Research Laboratory,
Canal Road, Jammu-Tawi

Introduction

PODOPHYLLOTOXIN since long has been known to possess medicinal properties and it is obtained from *P.emodi* Wall This crystalline substance was first isolated by Podwyssotzki(1). The alcohol soluble and water insoluble portion of podophyllum is a complex mixture containing most of biological activity (2) of original root It is usually called podophyllin but has also been named as podophyllum resin. The research on this drug was done on many fronts like pharmacology, biochemistry, cytology and clinical medicine by Kelly(3). The drug was studied in many clinical conditions including disease of skin due to infectious agents, non specific dermatoses, metabolic diseases, benign growth & malignant new growths. Podophyllin has been used in the treatment of cancer by private practitioners in US prior to 1897. It was reported by Hartwell(4) for the first time that podophyllin had destructive effect on cancer cells of experimental animals. It was demonstrated by King, L (5) that podophyllin produced its therapeutic effect by acting like colchicine as a mitotic poison. Podophyllin was so popular in the earlier days of last century as a cathartic and cholagogue that it was included in the first U.S. Pharmacopoeia (1820) and was listed until the twelfth revision published in 1942. Both American and Indian podophyllum were included in British Pharmacopoeia of 1948 but were dropped later from 1953 edition (6). American podophyllum has been listed at one time or other in most Europeon, South American and Asian Pharmacopoeia(7).

The chemistry and antineoplastic activity of many constituents of podophyllin were actively investigated at National Cancer Institute, USA by Hartwell J.L. in

the 1940's. A superb review of this later history, chemistry and pharmacology of podophyllum and its constituents was prepared by Hartwell and Schrecker(8). Its structure was proposed independently by Borche(9) and was later revised by Hartwell(10).

Podophyllin is an amorphous powder light brown to greenish yellow in colour. It has a characteristic odour, a bitter taste and is irritating to eyes and mucous membrane. Besides podophyllotoxin (I) and 4′-demethylpodophyllotoxin (II) podophyllin has other constitutents e.g. kaempferol, quercetin, podophyllotoxin-1-O-β-D-glucopyranoside (XVI), 4′-de-methylpodophyllotoxin-1-O- β-D-glucopyranoside,(XVII) and tannins. Extraction of podophyllin with chloroform yields a residue which upon recrystallisation from benzene:methanol and hexane gives a 9:1 mixture of podophyllotoxin and 4′-demethylpodophyllotoxin (30 to 40% based upon resin). The remaining chloroform insoluble portion is called the Marc which consists of quercetin, kampferol, tannins, podophyllotoxin-1-O-β-D-glucopyranoside(XVI) and 4′- demethylpodophyllotoxin-1-O-β-D-glucopyranoside(XVII).

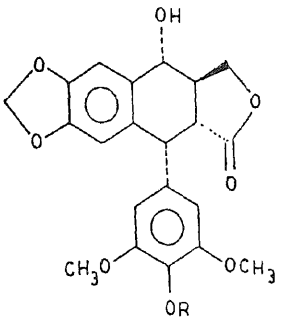
The introduction of anticancer drug Etoposide (XII) and Teniposide (XIII) has created a demand for podophyllotoxin, the natural tetralin lignan (podophyllotoxin) is used for the semi-synthesis of these drugs (12,13). Since the total synthesis of podophyllotoxin is still uneconomic, future production of these drugs will depend upon either systematic cultivation of Podophyllum plants or the use of tissue culture techniques(14) to obtain podophyllotoxins. Alternatively improvement in extraction techniques or new sources(15,16,17) of these compounds need to be exploited.

The lignans of podophyllum can be divided into: Podophyllum aglycones and Podophyllum glycosides

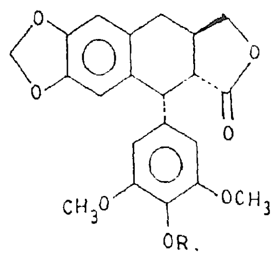
Podophyllum Aglycones

Podophyllotoxin (I), isolated as early as 1880 by Podwyssotzki, represents the main compound of Podophyllum species. Picropodophyllotoxin(XI) is easily formed by base catalyzed rearrangement of podophyllotoxin and was presumed to be an artefact(18) generated in the course of the isolation procedures. Desoxypodophyllotoxin (III) was isolated from all the Podophyllum species (17), it was known earlier also from the roots of *Anthriscus sylvestris* (Family Umbelliferae) by Kawanami(19) and was named "anthricin".

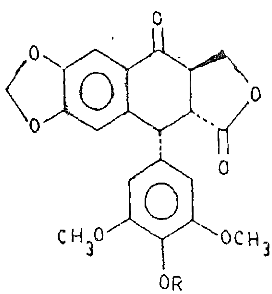
The earlier methods of isolation of natural products like extraction, fractional precipitation, crystallization have failed to yield pure compounds from *Podophyllum emodi* other than the two discovered by Podsyssotzki in 1880 namely podophyllotoxin and quercetin. In the span of ten years (1947-1955) the modern techniques of separation and purification such as column chromatography and thin layer chromatography yielded considerable number of additional substances such as α-peltatin (VIII), β-peltatin (VII), 4′-demethyl podophyllotoxin(II). The solvent



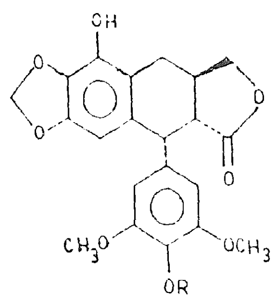
I, $R=CH_3$. Podophyllotoxin
II, R=H. 4'-Demethylpodophyllotoxin



III, $R=CH_3$. Deoxypodophyllotoxin
IV, R=H. 4'-Demethyldeoxypodophyllotoxin



V, $R=CH_3$. Podophyllotoxone
VI, R=H. 4'-demethylpodophyllotoxone



VII, $R=CH_3$. β-Peltatin
VIII, R=H. α-Peltatin

partition, and paper chromatography led to the separation of desoxypodophyllotoxin(III) and glucosides of podophyllotoxin and 4′-demethylpodophyllotoxin, β-peltatin and α-peltatin.

Commercial Method of Isolation of Podophyllotoxin

The commercial podophyllin is obtained by the extraction of powdered rhizome/roots of *P. emodi* with methanol or ethanol, followed by concentration under vacuum. This semisolid mass thus obtained is put into acidulated water (10ml)

hydrochloric acid into 1000 ml water cooled to 10°C slowly. The precipitate is allowed to settle, decanted and given washing with cold water. The resin upon drying gives a dark brown amorphous powder commonly called podophyllin. The podophyllin on extraction with chloroform and further purification by repeated recrystallisations from benzene alone or alcohol benzene mixture followed by thorough washings with petroleum ether/hexane yields the commercial podophyllotoxin The podophyllotoxin content of the commercial podophyllin usually ranges between 45 and 55%. The other compounds mostly present are identical with podophyllotoxin (See Table 1).

Dunstan and Henry (20) separated podophyllin (from *P.emodi* or *P.peltatum*) into three main fractions.

(A) Soluble in chloroform

(B) Insoluble in chloroform but soluble in ether

(C) Insoluble in chloroform and ether also called as podophylloresin

The chloroform soluble fraction was treated with boiling benzene and the solution cooled slightly, decanted from dark resin and allowed to stand for several days, during which podophyllotoxin separated slowly. Borsche and Niemann (9) employed same method to obtain podophyllotoxin which was then purfied by recrystallization from aqueous ethanol Later on Spath (21) improved the proceduce by dissolving the chloroform soluble fraction in ethanol and adding benzene: this made prior removal of the dark resin unnecessary. A recent preparative method by De-Ambresi (22) is based essentially on Spath's procedure. It is noteworthy that most of these preparations were carried out with Indian podophyllin which contains a much larger amount of podophyllotoxin and much smaller proportion of other lignans than American podophyllin.

In our laboratory we have further simplified and improved the method of extraction of pure podophyllotoxin by dissolving the chloroform soluble fraction in alcohol and refluxing it with neutral aluminium oxide so that the solution becomes light yellow in colour. To the alcohol solution added benzene which yielded podophyllotoxin of 95-98% purity. The function of the neutral aluminium oxide is to remove the polyphenolics such as kaempferol, quercetin and isorhamnetin and other minor polyphenolics present in the chloroform extract. Desoxypodophyllotoxin and other minor lignans could be removed by this method. We have experimentally proved that podophyllotoxin on refluxing with neutral aluminium oxide does not form picropodophyllotoxin(XI).

The commercial method of isolating podophyllotoxin from *P.emodi* roots/rhizomes earlier developed in this laboratory has also been modified. The improved method (29) of isolation of podophyllotoxin from commercial podophyllin/crude podophyllotoxin (the purity of which is 35 to 90%) involves extraction over a bed of neutral alumina with solvents such as benzene, toluene, xylene, dichloromethane etc The length of the bed of neutral aluminium oxide is the key factor of the process.

Table 1 : Lignan content percentage of Podophyllum & Diphylleia species

Lignan	*P.emodi** *P hexandru* root/rhizome	*P peltatum** *root/rhizom*	*P.pleianthium** root/rhizome	*P.versipelle** roots/rhizome	*D.cymosa**		*D.grayi**	
					root	leaf	root	leaf
Podophyllotoxin(I)	3-4	0.2	0.13	0.32	0.007	.54	1.2	.11
4′-Demethylpodophy-llotoxin(II)	0-4	0.06	0.041	0.014	0.004	.09	.07	.04
Desoxypodophy-llotoxin(III)	0-017	0.023	0.010	tr	-	-	-	-
4′-Demethyldesoxypo-dophyllotoxin(IV)	0-010	0.007	0.030	tr	-	.11	tr	-
Podophyllotoxone(V)	0-057	0.020	0.37	0.01	-	-	.12	-
4′-Demethylpodo-phyllotoxone (VI)	0-017	0 007	0.013	tr	-	-	.003	-
β-Peltatin(VII)	0-010	0.25 to 0.3	-	1.35	-	-	.12	tr
α-Peltatin (VIII)	0-007	0.20	-	0.12	-	-	tr	0.02
Isopicropodophyllone (IX)	0-033	0.007	0.020	-	-	-	-	-
4′-Demethylisopicro-podophyllone (X)	0.007	0.003	0.005	-	-	-	-	-
Podophyllotoxin-1-O-D-glucoside (XVI)	0-7*	-	0.058	.13	.13	.03	.13	.01

Contd.

(Contd.)

4'Demethylpodophyllo-toxins-1-O-Dglucoside (XVII)	0-08*	-	0.023	tr	-	-		-
β-peltatin- D-glucoside (XXII)	-	+	-	66	-	+	+	+
α-peltatin-5-0-glucoside (XVIII)	-	+	-	0 032	-	+	+	+

* Data from ref 16 & 17

* Data from ref. 33

+ Data from ref 15

tr = trace (less than 0.02 mg g^{-1} dry cut)

+ = present but not quantified

- = not detected

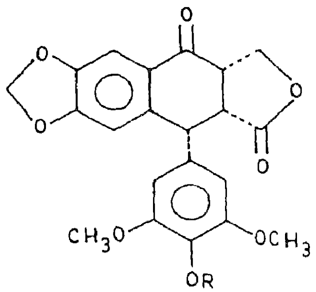
Figures in parentheses represent amount of aglycone after enzyme hydrolysis

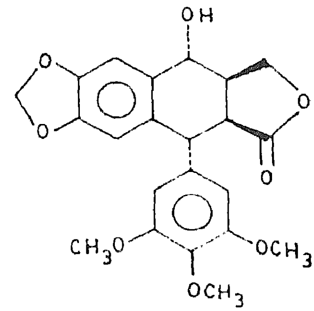
The function of aluminium oxide is to absorb the polyphenolics and tannins and also to act as a stationary phase to separate podophyllotoxin from other compounds present as impurities. The extraction may be done for a period ranging from half an hour to 4 hours. The extract upon concentration and recrystallization from organic solvent such as hot benzene/toluene yields pure podophyllotoxin (95 to 97%).

We have further developed (27) method of isolation of podophyllotoxin and 4′-demethylpodophyllotoxin from the chloroform and ether insoluble part also called marc which is a waste material of podophyllotoxin extraction process.The main constituents of the marc are the glycosides of podophyllotoxin and 4′-demethylpodophyllotoxin. The process consists of dissolving the marc in hot methanol and filtration. The filtrate is poured in water and emulsin (β-glucosidase) enzyme added to it. The mixture is kept at room temperature (30-37°C) with stirring and the resulting slurry is repeatedly extracted with chloroform at room temperature which upon concentration and recrystallisation yields a mixture of podophyllotoxin and 4′- demethylpodophyllotoxin in the ratio of 4:1 (total yield 0.6%) of Podophyllum roots/rhizomes. The process of the present invention increase the overall yield of the mixture of podophyllotoxin and 4′-demethylpodophyllotoxin.

The present observation provides a process for the isolation of a mixture of podophyllotoxin and 4′-demethylpodophyllotoxin which comprises treating powdered rhizomes with polar solvents, extracting with chlorinated solvents, separating the residue (marc) by known methods, hydrolysing the marc so obtained with enzyme emulsin in presence of polar solvents at a *p*H in the range of 5 to 7 at a temperature in the range of 30° to 37°C for 4 to 6 hours; extracting the mixture with chlorinated hydrocarbons treating with neutral alumina to get a mixture of podophyllotoxin and 4′-demethylpodophyllotoxin. The methods developed and described for the isolation of podophyllotoxin is either from the podophyllin, marc or resin of the podophyllum emodi roots. Keeping in view the lengthy and tedious steps, we further simplified the method of extraction of podophyllotoxin directly from the roots/rhizome of *P.emodi* (28).

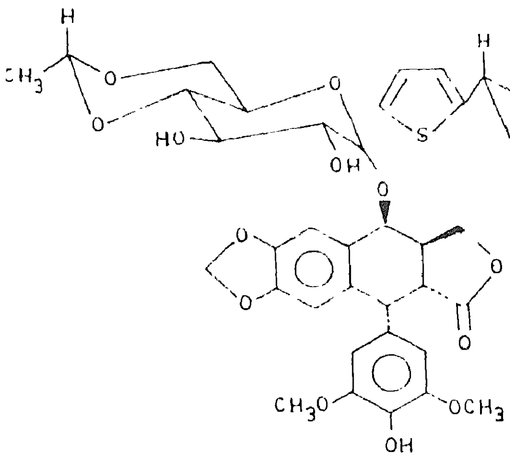
It is a single step process for the extraction of podophyllotoxin from the roots/rhizome of *P.emodi* . According to this method of isolation of Podophyllotoxin, the dry powdered roots/rhizomes of *P.emodi* are extracted with an organic solvent at a temperature in the range of 40-70°C over a bed of neutral aluminium oxide. The organic solvent used could be aromatic hydrocarbons such as benzene, toulene, xylene or chlorinated hydrocarbon such as dichloromethane, chloroform, 1,2-dichloroethane, carbon tetrachloride etc. The length of neutral aluminium oxide in the bed may depend upon the polarity of the organic solvent used and also on the type of polyphenolic content of *P. emodi*.The function of aluminium oxide is to absorb the polyphenolics and also act as the stationary phase to isolate the podophyllotoxin and 4′-demethylpodophyllotoxin and other aglycone compounds.



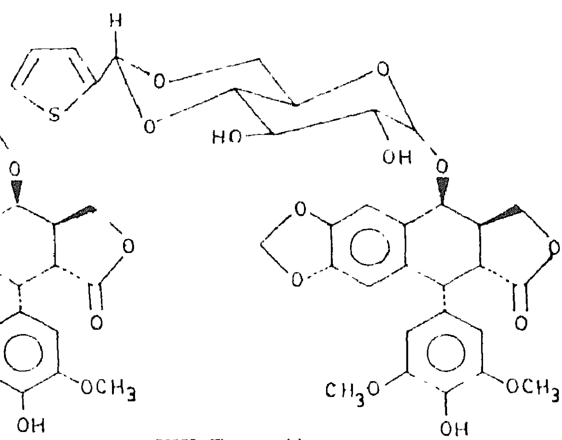


IX, R=CH_3. Isopicropodophyllone
X, R=H. 4'-Demethylisopicropodophyllone

XI. Picropodophyllotoxin



XII, Etoposide



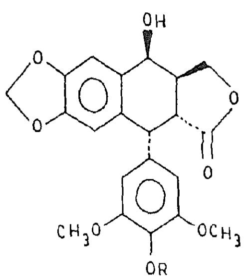
XIII, Temposide

The extraction is done for a period ranging from 16-36 hr The extract upon concentration and recrystallization from solvent such as hot benzene/toluene yields podophyllotoxin (2.5 - 3.5%). The purity of the podophyllotoxin has been determined by HPLC and found to be 97-98%.

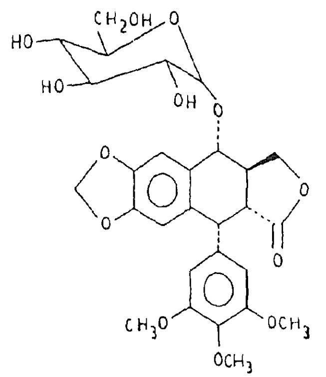
The method of isolation of podophyllotoxin developed in this laboratory is quite simple and involves isolation of required compound from the dry powdered roots in a single step without involving extraction with methanol/ethanol; concentration, treatment with water followed by drying in air and subsequent extraction with chloroform and concentration followed by repeated recrystallizations with benzene/methanol mixture

Podophyllum glycosides

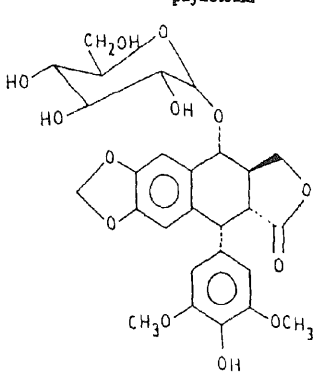
Nadkarni (23) in 1951 first reported the isolation of picropodophyllotoxin-1-O-β-D-glucoside (XVIII) from Indian podophyllin. Later on Wartburg et al., (24, 25, 26) isolated four new water soluble lignan glucosides from Podophyllum rhizomes.



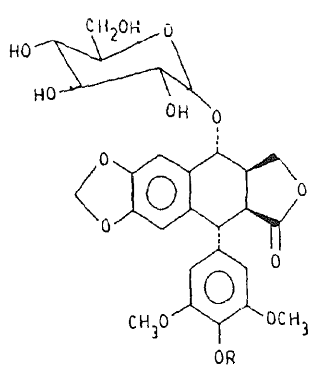
XIV, R=CH_3 Epipodophyllotoxin
XV, R=H 4'-Demethylepipodophyllotoxin



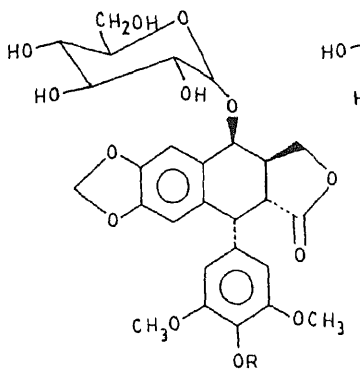
XVI, Podophyllotoxin-1-O-β-D-glucopyranoside



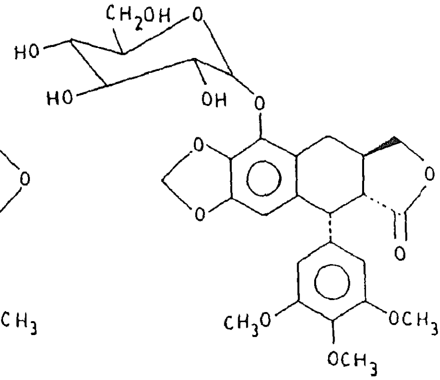
XVII, 4'-Demethylpodophyllotoxin-β-D-glucopyranoside



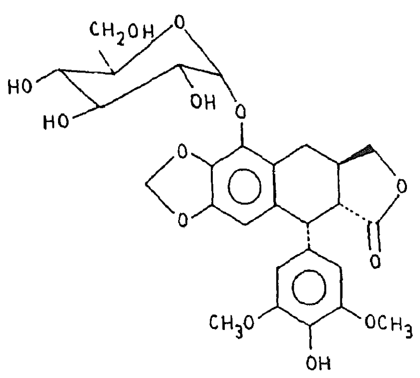
XVIII, R=CH_3 Picropodophyllotoxin-1-O-β-D-glucopyranoside
XIX, R=H 4'-Demethylpicropodophyllotoxin-1-O-β-D-glucopyranoside



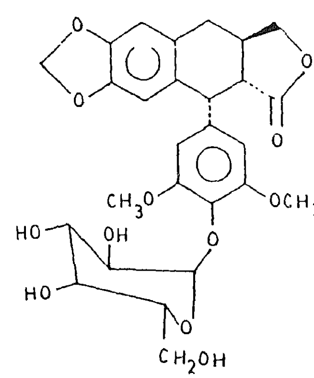
XX, R=CH_3, Epipodophyllotoxin-1-O-β-D-glucopyranoside
XXI, R=H, 4'-Demethylepipodophyllotoxin-1-O-β-D-glucopyranoside



XXII, β-Peltatin-β-D-glucopyranoside



XXIII, α-Peltatin-β-D-glucopyranoside



XXIV, 4'-Demethyldesoxypodophyllotoxin-4'-β-D-glucopyranoside

In this method the dried roots/rhizomes of *Podophyllum peltatum* were extracted with 90% methanol, and the solution was concentrated, diluted with water and extracted with chloroform. After precipitation of tannins with lead acetate; it was re-extracted with chloroform and chloroform butanol (9:1 then 7:3). The various extracts were placed on column of dry or water saturated diatomaceous earth in the order of decreasing partition coefficient (ethyl acetate/ water); the following

Table 2: Physical constant and LD_{50} of Podophyllotoxin lignans & their glycosides

Lignan/glycoside	Composition	m.p.	{a}D	Uv EtOH men (20)	LD-50* mouse mg/kg
Podophyllotoxin(I)	$C_{22}H_{22}O_8$	183-184°	-133°($CHCl_3$)	281 logE(3.63)	35
4'-Demethylpodo-phyllotoxin(II)	$C_{21}H_{20}O_8$	250-252°	-130°($CHCl_3$))	291(3.64)295 sh	>120
Desoxypodophy-llotoxin(III)	$C_{22}H_{22}O_7$	167-168	-116 ($CHCl_3$)	282 log (3.64)	52
4'-Demethyldesoxypo-dophyllotoxin(IV)	$C_{21}H_{20}O_7$	234-237	-	291 (3.61) 296sh	
Podophyllotoxone(V)	$C_{22}H_{22}O_8$	191-192	-123°($CHCl_3$)	271,280 sh	
4'-Demethylpodo-phyllotoxone(VI)	$C_{22}H_{20}O_8$	173-185	-	282,287,293,297sh	-
β-Peltatin(VII)	$C_{21}H_{20}O_8$	232-235	-125($CHCl_3$)	273,313	-
α-Peltatin(VIII)	$C_{22}H_{22}O_8$	230-232	-123°($CHCl_3$)	272,280 sh	
Iso-picropodophyllone (IX)	$C_{22}H_{22}O_8$	-	-	-	-
4'-Demethylisopicro-podophyllone(X)	$C_{21}H_{20}O_8$	-	-	-	-
Picropodophyllotoxin (XI)	$C_{22}H_{20}O_8$	225-227	+9°($CHCl_3$)	278,294 sh	-

Contd.

(Contd.)

Epipodophyllotoxin++ (XIV)	$C_{22}H_{22}O_8$			
4′-Demethyl-++ epipodophyllotoxin (XV)	$C_{21}H_{20}O_8$	228-30°C	-69.8($CHCl_3$)	244,249
Podophyllotoxin-β-D glucopyranoside(XVI)	$C_{28}H_{32}O_{13}$	152-154°	-76° (MeOH) 297	
4′-Demethylpodo-phyllotoxin-β-D- Glucopyra-noside(XVII)	$C_{27}H_{30}O_{13}$	165-170°	- 81° (MeOH)	
Picropodophyllotoxin -α-D-glucopyranoside (XVIII)	$C_{28}H_{32}O_{13}$	237-238°	- 11°(Pyridince)	
β-Peltatin-β-D glucopyranoside(XXII)	$C_{28}H_{32}O_{13}$	154-156	-123°(MeOH)	200
α-Peltatin-β-D glucopyranoside(XXIII)	$C_{27}H_{30}O_{13}$	168-171	-129°(MeOH)	200
4′-Demethyldesoxy-podophyllotoxin-4′-β-D glucoside(XXIV)	$C_{27}H_{30}O_{12}$	-	-	400

* LD-50 dose killing 50% of animals upon single administration;
application was i.v. or i.p. ; no major difference being between these two routes where both were tested.

\+ Data from ref. 8

* Data from ref. 32

++ Data from ref 34

fractions were eluted with moist ethyl acetate (a) aglycones (b) the glucosides of podophyllotoxin (XVI) and β-peltatin (XXII, partial separation) (c) the glucosides of 4′- demethylpodophyllotoxin (XVII) and α-peltatin (XXIII). Complete separation of the glucosides was then accomplished by chromatography on silica gel and fractional elution with moist isopropyl acetate containing 0.5 to 2% methanol. By essentially the same procedure, the glucosides of podophyllotoxin (XVI) and 4′-demethylpodophyllotoxin (XVII) were separated from *P. emodi.*

We have observed that the Indian *P. emodi* in addition to the podophyllotoxin 1-O-β-glucopyranoside(XVI) and 4′- demethyl-podophyllotoxin-1-O-β-D- glucopyranoside(XVII) also contains the Picropodophyllotoxin- β-D-glucopyranoside (XVIII) and 4′-demethylpicropodophyllotoxin-- D-glucopyranoside (XIX). In the American species *P. peltatum*, *Linium flavum* (15) and *Linium capitatum*(17) β-peltatin-β- D-glucopyranoside (XXII) and α-peltatin-β-D- glucopyranoside (XXIII) are present.

Our laboratory has developed (30) a process for the extraction of glucoside of podophyllotoxins from the marc (material left after the extraction of podophyllotoxin and 4′-demethyl-podophyllotoxin from the resin) or insoluble part in chloroform and ether. The marc which comprises of kaempferol, quercetin, podophyllotoxin-1-O-β-D-glucopyranoside (XVI) and 4′-demethylpodophyllotoxin-1-O-β-D-glucopyranoside (XVII) was dissolved in hot methanol which gave a dark brown solution decolourised by refluxing the solution with neutral aluminium oxide repeatedly to yield a light yellow solution. This solution upon concentration under vacuum gave light yellow amorphous solid, HPLC analysis of solid indicated it to be a mixture of podophyllotoxin-1-O- β-D-glucopyranoside(XVI) and 4′-demethylpodophyllotoxin-1-O-β- D-glucopyranoside (XVII) in the ratio of 9:1. The total yield of podophyllotoxin 1-O- β-D-glucopyranoside and 4′-demethylpodophyllotoxin-1-O-β- D-glucopyranoside varies from 0.8-1.2% in *P.emodi* marc. The method of extraction of the mixture of glucosides of podophyllotoxin is quite simple and does not involve the extraction with lead acetate (in order to precipitate tannins). The process reported in literature (31), claims that the glucosides are water soluble. The observation has been varified by extracting water (in which alcoholic extract has been poured) with n-butanol. It showed the presence of trace amounts of these glucosides upon HPLC analysis showing that the major amount of these glucosides remained in marc only. The identity of glycosides was established both by hydrolysis with emulsin (17) as well as by running standard samples of podophyllotoxin-1-O-β-D- glucopyranoside and 4′-demethylpodophyllotoxin-1-O- β-D-glucopyranoside on HPLC.

Accordingly, the present improved process developed by us for the isolation of a mixture of podophyllotoxin-1-O-β- D-glucopyranoside and 4′-demethylpodophyllotoxin-1-O-β- D-glucopyranoside from *Podophyllum emodi* comprises dis-

solving powdered rhizomes of *P. emodi* in polar solvents to get extract, concentrating the extract and mixing with ice cold water and isolating the resin (residue) so obtained, drying of the resin, extracting with an organic solvent, separating the marc left as residue by known methods, treating the said marc with polar solvent, separating the filtrate and refluxing repeatedly with neutral alumina, removing the solvent by known method to get a mixture of podophyllotoxin-1-O-β-D-glucopyranoside and 4′-demethylpodophyllotoxin-1-O-β-D-glucopyranoside. The polar solvent used is selected from methanol, ethanol, propanol, isopropanol etc. The concentration of extract is effected under vacuum. The chlorinated solvent used is selected from dichloromethane, chloroform, carbon tetrachloride, dichlororoethane etc. An example of the process is given as follows.

Air dried powdered roots/rhizomes of *P. emodi* (100g) were extracted with methanol (500 ml) in a soxhlet for 15 hr. at a temperature in the range of 50-55°C. The extract concentrated under vacuum and poured in ice cold water (500 ml) with constant stirring. The water removed by decantation. The resin was washed with water (3×200 ml) and dried at room temp. when it gave light brown coloured powder (9.5 g). It was extracted with chloroform (7×50 ml) which on concentration and repeated crystallization with benzene:methanol gave a mixture consisting of podophyllotoxin and 4′-demethylpodophyllotoxin in the ratio of 9:1 (3.5 to 4 gms) and light brown coloured marc (4.0 g). The composition of the marc is kaempferol, quercetin, podophyllotoxin-1-O-β-D-glucopyranoside and 4′-demethylpodophyllotoxin-1-O-β-D-glucopyranoside. The colouring matter like polyphenolics were removed by dissolving the marc in methanol (25 ml) and filtration. The filtrate refluxed repeatedly with neutral alumina (5×4g). The methanol extract on removal of solvent gave a solid which was recrystallised from aqueous methanol to give amorphous powder (0.8g- 1.2g). The HPLC analysis of the product showed it to be a mixture of two compounds namely (a) podophyllotoxin -1-O-β-D-glucopyranoside and (b) 4′-demethylpodophyllotoxin-1-O-β-D-glucopyranoside in the ratio of 9:1.

The methods developed by Wartburg and other are very lengthy, tedious and involve mixture of solvents. The methods developed by us from the total extract and marc also involve number of steps. In order to improve upon these methods we have further modified the method of isolation of glycosides of podophyllotoxin from the roots/rhizome directly.

The method developed is very simple and economical for the isolation of the podophyllotoxin glucosides and 4′-demethylpodophyllotoxin glucoside from the roots/rhizome of *Podophyllum emodi*. In this method first of all the aglycones of podophyllotoxin and 4′-demethylpodophyllotoxin and other minor lignans such as podophyllotoxone, desoxypodophyllotoxin, 4′-demethylpodophyllotoxin etc. are removed by the extraction with organic solvents such as benzene, chloroform,

dichloromethane, hexane etc. The plant material left after the removal of these aglycones is extacted with polar solvents like ethyl acetate, acetone or alcohols. The polar solvent extract is treated with neutral metal oxide such as neutral aluminium oxide. The neutral aluminium oxide absorbs most of the polyphenolics. The glucosides of the lignan are filtered and concentrated in vacuum. This extract generally contains trace amount of aglycones of podophyllotoxins whereas podophyllotoxin glycoside in major quantity and 4′-demethylpodophyllotoxin glycoside in minor quantity along with the trace amount of the picro- podophyllotoxin-1-O-β-D-glucopyranoside (XIV) and 4′- demethyl-picropodophyllotoxin-1-O- β-D-glucopyranoside(XV). The separation-purification of these glycosides are done on silica gel column to yield the pure podophyllotoxin-1-O-β- D-glucoside and 4′-demethylpodophyllotoxin-1-O-β- D-glucoside. The column was eluted with chloroform: methanol (95:5).

Fraction A: yields the mixture of aglycones of podophyllotoxin, 4′-demethylpodophyllotoxin and podophyllotoxone.

Fraction B: on elution with chloroform : methanol (9:1) yields mainly podophyllotoxin-1-O-β-D-glucoside.,

Fraction C: on elution with chloroform: methanol (85:15) yields 4′-demethylpodophyllotoxin-1-O-β-D-glucoside.

On technological side the earlier methods were tedious, cumbersome and lengthy involving number of steps. The methods developed by us for the isolation of podophyllotoxin are performed in single step and subsequent crystallisation processes yields pure compound. However the glycosides are also extracted from the plant in a single step. The separation and purification has been done on column chromatography. The products are quite pure and the process is economical.

Podophyllotoxin is a relatively rare natural product and is presently extracted from the roots/rhizome of two species of Podophyllum (Berberidaceae) *P. emodi* Wallich/*P.hexandrum* (Indian podophyllum) and *P. peltatum* (American podophyllum). The former species is much richer source of podophyllotoxin containing upto 4.0% with *P. pellatum* yielding near of 0.25%. The Chinese species *P. versipelle* and two species of Diphyllcia, *D. cymosa* and *D. grayi*, classified in family berberidaceae sub family podophylloideae; contain podophyllotoxin ranging from 0.2 to 1%.

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Isolation and Evaluation of Valepotriates from Indian Valerian

B.K.Gupta, J.L.Suri and G.K.Gupta

Regional Research Laboratory, Jammu

Introduction

VALERIANA Linn.[1] (N.O.Valerianaceae) is a genus of perennial herbs or undershrubs. Of about twelve species occurring in India only three namely *Valeriana hardwickii, V. officinalis* and *V. jatamansi* or *V. wallichii* are considered important. The dried rhizomes and roots of *V. jatamansi* (Mushkbala in Hindi) have been recognised as Indian Valerian in IPC. It is found in the temperate Himalayas at an altitude of about 3,000 m and in the Khasi and Jaintia hills between 1,500 m and 1,800 m. It is abundant in the Western Himalayas and easily responds to cultivation. It prefers deep rich soil and flourishes in shady and moist localities.

Indian Valerian is prescribed as a remedy for hysteria, hypochondriasis, nervous and emotional troubles. It is also used as carminative and forms an ingredient of a number of Ayurvedic recipes. Before the discovery of Valepotriates, the essential oil[2] from *Valeriana* roots was considered as the most active principle especially the compound bornyl isovaleriate. Later studies have shown that essential oil has a very small sedative effect. The therapeutic properties of Valerian are due to a group of iridoids called Valepotriates which are useful as tranquilizers and sedatives.

Valepotriates were first isolated by Thies and Funke[3] in 1966 from *Valeriana wallichii* DC. This was followed by investigations by a number of groups on various species of *Valeriana* in a number of countries particularly in Germany[3-10], Bulgaria[11-14], Netherlands,[2,15-17], Denmark[18], Poland[19] and Russia[20,21]. Valepotriates

are triesters of a polyhydroxy alcohol derived from the terpenoid 4,8-dimethylcyclopenta (c) pyran with isovaleric, acetic, isocarproic and β-acetoxy isovaleric acids. However, the alcohol does not exist in nature. They are called iridoids because they all contain a cyclopentanopyran ring system which is related to the possible parent compound iridodial (Fig. 1).

According to Thies, the Valepotriates are divided into two groups depending on their colour reactions.

1. The group of conjugated dienes which with acids (HCl + CH_3COOH) give blue salts soluble in water.
2. The group of monoenes which react with non-oxygen acids and give a non-specific brown colouration.

The Valepotriates are unstable and undergo rapid decomposition at temperature > 40°C and are destroyed by mineral acids (pH < 3) and alkalis (pH > 11).

The main valepotriates isolated from *Valeriana wallichii* are valtrate, isovaltrate, homovaltrate, acevaltrate, homoacevaltrate and IVHD-valtrate (Fig 2). All these compounds degrade to yield baldrinal, homobaldrinal and their corresponding acids depending on the nature of the compounds. The structure of Valepotriates and their decomposition products (Fig. 2).

Baldrinal, R_3 = Ac
Homobaldrinal, R_3 = IS

	R_1	R_2	R_3
Valtrate	IS	IS	Ac
Isovaltrate	IS	Ac	IS
Homovaltrate	IS	IC	Ac
Acevaltrate	IS (or R_2)	ISAc (or R_1)	Ac
Didrovaltrate	IS	IS	Ac
Didroisovaltrate	IS	Ac	IS
Homodidrovaltrate	IC	IS	Ac
IVHD-Valtrate	IS (or R_2)	ISIS (or R_1)	Ac
Homoacevaltrate	ISAc	IC	Ac

Where IS = $-COCH_2CH(CH_3)_2$

Ac = $-COCH_3$

IC = $-CO(CH_2)_2CH(CH_3)_2$

ISAc = $-COCH_2C(CH_3)_2$ with $OCOCH_3$ on the C

ISIS = $-COCHCH(CH_3)_2$ with $OCOCH_2CH(CH_3)_2$ on the CH

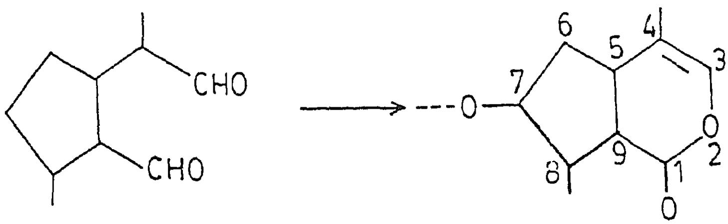
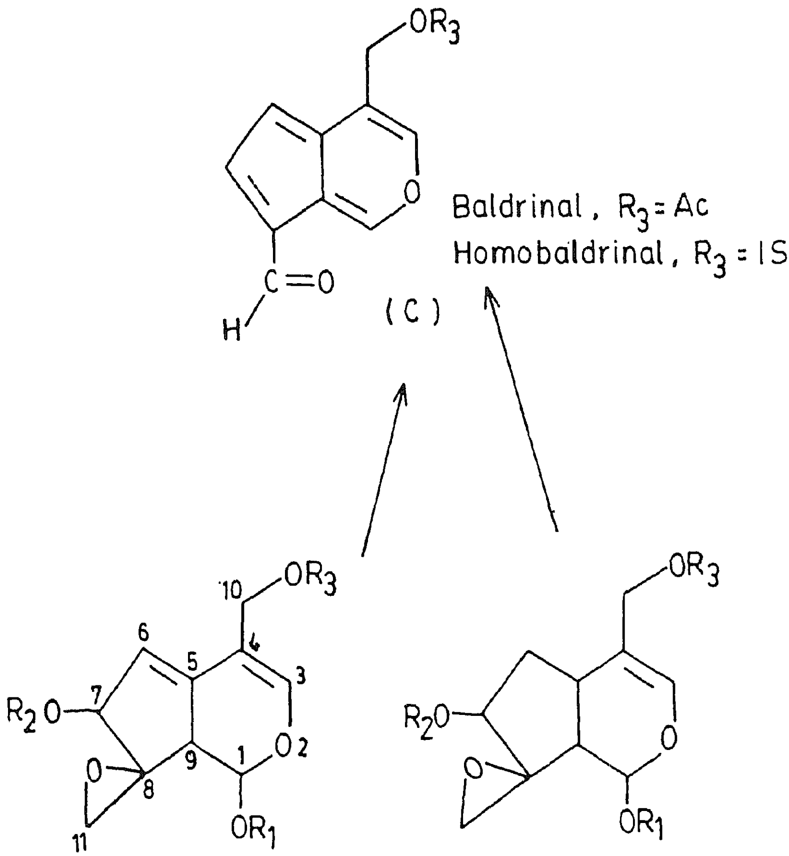

CHO
CHO
O
6
5
4
3
7
O
2
8
9
1
O

FIG. 1


OR3
O
C=O
H
(C)
Baldrinal, R3 = Ac
Homobaldrinal, R3 = IS
10
OR3
6
5
4
3
R2O
7
O
O 2
9
1
8
11
OR1
OR3
R2O
O
O
OR1

(A) (B)

FIG. 2

Isolation of Valepotriates

Keeping in view their highly sensitive nature, very advanced research and production facilities are required for the commercial production of these compounds. At present these compounds are exclusively being produced by Kali-Chemie of Germany. A number of patents[31-34] for the production of these compounds have been taken by P.W. Thies of the said Company. A survey of the literature showed that no work has been done so far in India on the isolation of these tranquilosedating agents from Indian Valeriana and most of this drug is exported to Europe for lack of technical know-how for its processing in the country. An exhaustive investigation by the authors has resulted in the development[25] of an economical method for the production of Valepotriates from Indian Valeriana, a summary of which is given below.

The drug is dried at a temperature not exceeding 35°C and carefully powdered avoiding excessive heating during the operation. The powdered drug is stirred with methylene chloride and allowed to stand overnight at 30°C. It is then transferred in a percolator with an additional quantity of solvent and percolation started. The process was repeated thrice. The dark brown percolate is clarified with charcoal yielding a light yellow solution which is washed successively twice with half its volume of 3% sodium bicarbonate and twice with half its volume of 5% sodium chloride solution (to remove free acids). This is then dried over anhydrous sodium sulphate and concentrated in the rotary film evaporator at 30°C to about one tenth of its volume. The concentrated solution is washed again successively with 0.1% sodium hydroxide and 5% sodium chloride, dried over anhydrous sodium sulphate and further concentrated untill all the solvent is removed yielding a light yellow oily product of about 50% purity (calculated as valtrate). The yield of the oily product on the basis of dried drug is about 2.4%.

Evaluation of Drug

The contents of Valepotriates vary depending upon the variety, origin, time of collection and the method of drying the herb. Many European varieties of *Valeriana* do not contain any Valepotriates at all. The quantitative ratios of the three main Valepotriates are also different in plants of different varieties. For example, Valtrate clearly dominates in *Valeriana officinalis*, whereas the above mentioned three compounds (Valtrate, acevaltrate and didrovaltrate) are almost in equal quantities in *V. wallichii*. This enables to chemically differentiate the two varieties and identify the herb for study. The most important step in the evaluation of the drug is qualitative and quantitative determination of the valepotriates content. A number of methods are known in the literature for the quantitative determination of Valepotriates in the drug and its preparations. The estimation is often made either by spectrophotometry or colorimetry. Colorimetric[9,22,23] and titration[24,25] methods can be used to evaluate the total esters present in the sample. UV[19] method of

analysis can measure only the dienes present in the sample but to determine quantitatively each component HPLC[2,17,26-30] is the method of choice. The detailed procedure of UV and colorimetric methods are given below:

(a) U.V. Spectrophotometry

The derivatives that absorb in UV (Valtrate and acevaltrate with conjugated diene system λ_{max}^{256} are estimated by UV spectrometry after chromatographic separation and eluation of the spots.

A known volume (0.03 ml) of extract is applied on TLC plates coated with silica gel GF 254. The chromatograms are developed for 15 minutes by using a mixture of hexane methyl ethyl ketone (8:2) dried at a temperature of 20°C and observed in UV 254 nm light. The spots of fluorescence-suppressing substances (which appear as blue spot on green ground coat) are marked with a needle and the marked zones of adsorbent are transformed quantitatively to a small filtrate made of sintered glass G-4 and eluated with 3 portions of methanol and volume made upto 10 ml. Absorption of the solution is measured at 256 nm and the valtrate content is determined by using the coefficient $E_{1\%}^{1cm} = 379$. This method gives an accuracy of ± 8%.

(b) Colorimetry or Densitometry

The technique is based on the highly exhibited coloration reaction and was developed by Wagner *et al.*[22]. The reaction principle consists in the formation of a hydroxamic acid by hydroxylamine in highly alkaline medium by the rupture of ester function according to the following scheme:

$$\begin{array}{ll} RCOO - R' + NH_2OH \ \text{-------} & RCO - NHOH + R'OH \\ & \ | \\ & RC = NOH \\ & \ | \\ & OH \end{array}$$

The hydroxamic derivatives on that are formed, give in acid medium, a coloured ferric complex with $FeCl_3$ of the formula, $(R\text{–}CO\text{–}NHO)_3Fe$ whose intensity of colouration is proportional to the quantity of the hydroxamate formed and therefore to that of the ester in question.

The method consists in subjecting the dichloromethane extract of drug to TLC as described earlier. The individual zones treated with standard solutions of alkaline NH_2OH followed by acidic $FeCl_3$ (in absolute ethanol or methanol) and resulting colour determined at 512 nm. Pure compounds are used as reference.

Densitometric Method

This method is a modification of Wagner's colorimetric method and was first developed by J.P.Chapelle[23]. The densitometric method enables us to estimate the

isolated spot without the risk of interference. It is independent of temperature and is reproducible. The error is less than 6%.

Titration Method

This method is useful in the quantitative estimation of total esters in a sample. A modified method[35] developed by the authors consists in dissolving a known weight (W) of the extract in a known volume (V_1) of methanol. An aliquot volume (V_2) of this solution is neutralized with 0.01N NaOH using phenophthalein as indicator. Subsequently 10 ml more of 0.01N NaOH are added and contents heated as 56-59°C for 3 min. diluted with 200 ml of water, cooled and titrated with 0.01N H_2SO_4. From this, the amount of alkali consumed for the hydrolysis of the esters is obtained and percentage of total Valepotriates in terms of valtrate is obtained by the following relation:

$$\text{Percentage of total (V.P.)} = \frac{0.00106 \times V_1 \times V_3 \times 100}{W \times V_2}.$$

V_1 = Total volume of the extract.

V_2 = Aliquot volume of the solution used.

V_3 = Volume of 0.01N NaOH consumed.

W = Weight of extract.

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Effect of Light vs Dark on Seed Germination of *Podophyllum hexandrum* Royle

D.K.Chaudhary, B.L.Kaul & S.Khan

Regional Research Laboratory,
Jammu-Tawi

Introduction

PODOPHYLLUM HEDANDRUM Royle (Syn. *P. emodi* Wall) is an important medicinal plant. It is an erect glabrous succulent herb with creeping perennial rhizomes. This plant occurs at high altitude in inner ranges of Himalayas usually at 3000-4000m. Requires moist shady places rich in humus and decaying organic matter. Rhizomes and roots constitute the drug-Indian Podophyllum which yield medicinal resin or podophyllin finding use in a variety of ailments including cancer. Anticancer drugs viz., Etoposide and teniposide have been developed by conversion of major lignan podophyllotoxin from the resin[1]. *P. hexandrum* contains three times podphyllum resin than the American species *P. pelltatum*[2]. Besides, because of the intense collection of this plant from the forests and the lack of organized cultivation, *P. hexandrum* has been declared a threatened species[3]. Moreover, cultivation of this crop is presently difficult task, because under natural conditions seeds have been found to germinate after remaining dormant for one or two years[4-5]. Thus the present study was undertaken to break the seed dormancy of this species. The results achieved are being communicated here.

Materials and Methods

Three months old seeds of *P. hexandrum* (2n = 12) collected from Rambara, Chamoli (U.P.) were used in this study. Dry seeds were sterilized with Mercuric chloride (0.2% ; 20+°C) for 8 min., followed by rinsing with sterilized distilled water for 30 min., seeds were then immediately put for germination on thick moist filter sheets in Petridishes and were allowed to germination at room temperature (10 to 22°C) during January-March, 1994. One set of petridishes was placed under continuous dark conditions and other set under fluorescent light conditions (10 hrs light, 14 hrs dark), where filter sheets were regularly watered with sterilized distilled water to avoid any contamination. Since seeds did not germinate upto 25 days of sowing, ungerminated seeds were washed with sterilized water and scarified with a sharp teasing needle removing the sections of seed coat, with two to three incisions given around the hilum region, thoroughly washed with sterilized distilled water and then maintained under dark and light conditions at room temperature. Germination was considered to have occurred when radical emerged. Counts were made at regular intervals, the mean percentage of germination was worked out at the end of the experiment.

Results and Discussion

Under dark conditions, scarification of section of seed coat resulted in seed germination, after 30 days of sowing and maximum germination (60.0%) was recorded between 45-60 days of sowing. Whereas, under light conditions germination was very poor (8.89%) during this period. However, in control (unscarified) absolutely no germination was noticed even after 120 days of sowing. Nevertheless, 78.88% and 14.44% germination was recorded after 120 days under dark and light conditions respectively. Thus dark treatment gave 445.0% increase in germination over light treatment, at the end of the experiment. (Table 1). So far we have tried the effect of several, growth regulators, such as thiourea, IAA, GA, ethephon, chilling treatment etc.[6] to overcome the poor and erratic germination of this species. But, none of the treatments except seed scarification and dark treatment gave such

Table 1: Effect of light vs dark on seed germination (%) in *P. hexandrum*

Treatment	Germination (%) after							
	30	45	60	75	90 (Days)	105	120	Total Germination %
Light	0.00	5.56	3.33	1.11	1.11	0.00	3.33	14.44
Dark	1.11	18.89	41.11	4.44	4.44	8.89	0.00	78.88
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

a high, quick and uniform germination within shortest period of 45- 60 days. Morever, the evidence so far available shows that time dependent seed dormancy does not play any significant role in poor and erratic germination of *P. hexandrum*. The principal source of hindrance seems to be hard seed coat, which causes failure of radical to emerge and dormancy factor(s) may reside in the endosperm and or the seed coat[7].

Acknoweldgement

Thanks are due to Dr.R.S.Kapil, Director, Regional Research Laboratory, Jammu for his keen interest and facilities provided for these studies. We are also grateful to Dr.Virendra Singh for providing us authentic seeds of *P. hexandrum* for this study.

Summary

Effect of light vs dark seed germination of *Podophyllum hexandrum* Royle is reported. Scarification of seeds and dark conditions during germination favours quick and uniform seed germination of this species as compared to light conditions.

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Coleus forskohlii (Willd.) Briq. — An Overview

Virbala Shah

Hoechst India Limited
Centre For Basic Research
Bombay - 400 080

Introduction

COLEUS FORSKOHLII (Willd.) Briq., [synonym *C. barbatus* (Andr.) Benth.] belonging to the family Labiatae (Lamiaceae) is an ancient root drug recorded in Ayurvedic materia medica (Raj Nighantu, ca. 1340; Nighantu Ratnakar, 1867; Shaligram Nighantu, 1896, and Raj Nighantu Samhito Dhanvantariya Nighantu, 1896) under the Sanskrit name "Makandi" and "Mayini". The drug Makandi is described in these works as multiple fragrant tuberous root, bitter, sharp and sweet to taste growing on hills. The drug is claimed to improve appetite, facilitate digestion, increase vitality and is useful in anaemia, inflammation, flatulence, dropsy, fever with rigors, infestation of worms, spleenomegaly, dysentery, chronic abdominal problems, colitis and piles. The "vaidyas" practicing ayurvedic system of medicine are, however, unfamiliar with the therapeutic properties of this drug, and as a consequence it is unavailable in the herbal drug stores in India. Several ethnomedicinal uses of root tubers and leaves of *C. forskohlii.*, for human as well as veterinary ailments are noted in nature and literature. Root juice is given to children in constipation (Singh *et al.*, 1980). Kotas, the native tribals of Trichigadi in Nilgiris, South India consider decoction of tubers as tonic (Abraham, 1981). Roots are eaten for cough in Kumaon Himalayas, and 1-3 teaspoonful of root decoction is recommended for treatment of asthma in Maharashtra. Roots, in Maharashtra and Gujarat are believed to have blood purifying action (Shah, 1989).

Varma *et al.*, (1990) have reported following different uses of tubers - they are used as an anthelmintic; a few drops of root extract in milk are administered to infants to relieve constipation and abdominal pain, and about 2g of tubers are chewed by adults for abdominal pain. Application of the paste of fresh tuber to the festering boils to allay burning sensation, and paste mixed with mustard oil for skin infection and eczema is recommended by the natives in the Kumaon Himalayas (de Souza and Shah, 1988). Leaves are indicated for stomach problems (Varma *et al.*, 1990). Amongst the veterinary uses, administration of fresh tubers, raw or cooked, mixed with feed to the ailing cattle suffering from loss of appetite, distended stomach and sluggishness is a common practice in Kumaon Himalayas, Gujarat (de Souza and Shah, 1988), Maharashtra and Karnataka (Shah, 1989). Root tubers mixed with milk is administered to stimulate breeding bulls in Methujani betta in Coimbatore District, Tamil Nadu (Narayanaswami, 1930). Application of paste of fresh tubers to cattle skin in alopecia and use of fresh half-cooked leaves as galactogogue are the field reports from the Kumaon Himalayas (de Souza and Shah, 1988).

Outside India, the plant is reported to be of medicinal value in Zanzibar and Pemba; as an expectorant, emmenagogue and diuretic in Egypt and Arabia, and for liver fatigue and intestinal disorders in Brazil (de Souza and Shah, 1988; Kelecom, 1983). Boiled leaves are used as febrifuge and sap is taken for stomach trouble in Uganda (Richards, 1965). "Caboclos" tribe in Amazonia considers leaves to be analgesic (Elisabetsky and Castilhos, 1990).

The species is being cultivated to a limited extent for root tuber in the States of Gujarat, Maharashtra, and Karnataka in India. During season, root tubers are available in the vegetable markets as "Garmar" in Gujarat (April-June), "Mainmool" or Garmar in Maharashtra (April-June), and "Mammool" or "Magniberu" in Karnataka (November-January) where they are used for preparing pickles in households and also by industry.

Pharmacological studies of *C. forskohlii* (Bhakuni *et al.*, 1971; de Souza, 1977) revealed cardiovascular activity in the root extract. Activity guided fractionation of the alcoholic extract led to isolation of an active diterpenoid named Forskolin (Coleonol) (Dubey *et al.*, 1974; Bhat *et al.*, 1977). Further pharmacological and biochemical investigations established that forskolin possesses multifaceted biological activities such as positive inotropic, antihypertensive, bronchospasmolytic, antithrombotic, platelet aggregation inhibiting, antiglaucoma and adenylate cyclase stimulation (Rupp *et al.*, 1986). The novel feature of forskolin is its unique mechanism of generating cyclic AMP in the cells through direct activation of the catalytic unit of adenylate cyclase enzyme (Metzger and Lindner, 1981; Seamon *et al.*, 1981; Seamon and Daly, 1981a). This hitherto unknown mechanism of the compound opened up vistas for the biomedical research community to understand the CAMP mediated physiological processes. The intensity of global interest in forskolin compound could be judged from the fact about 600 research publications on forskolin have appeared in a short span of 6-7 years since 1981 (de Souza and

Shah, 1988). Growing demand for forskolin as a research tool and its potential as a therapeutic agent attracted chemists to synthesise the molecule, and botanists and phytochemists to search for alternate and richer plant source of forskolin in related species and genera. These exploratory efforts have resulted in confirming *C. forskohlii* , as, so far, the only species to contain forskolin. Thus *C. forskohlii* , came into prominence as an exclusive source of forskolin. Consequently, cultivation of the plant was taken up by the industries and entrepreneurs. Simultaneously, agriculturists and plant breeders took up improvement of the *Coleus* varieties for increased forskolin content and enhanced root-mass productivity (de Souza, 1991). This paper reviews literature on *C. forskohlii* and also provides some first hand information, particularly on its botany and agronomy.

1. Botany

1.1 Taxonomy

The genus *Coleus* was conceived by J. Loureiro in 1790 for the species *C. amboinicus* in his "Flora Cochinchinensis". The genus *Coleus* derives its name from a Greek word "koleos" meaning sheath referring to the sheath-like dorsally open monoadelphous stamens united at the base. The character conveyed by the generic name is also the character of circumscription of the genus and segregates genus *Coleus* from its allied earlier published genus *Plectranthus* having free stamens. Presently, there is no unanimity regarding generic status of *Coleus* Taxonomists (Morton, 1962; Willemse, 1979) working on the African flora observing varying degree of fusion of the stamens in certain populations of *Coleus* disagreed to accept the character of monoadelphous stamens as a reliable character and merged all the *Coleus* spp. under a larger generic concept of *Plectranthus*. Cramer (1978) in revision of Sri Lankan *Coleus* , however, upheld connate filaments to be a reliable character and so is the genus *Coleus*. Species of *Coleus* growing over their range of distribution in the Indian subcontinent have been examined by the present author and found consistently to show distinct character of connate filaments. In view of this, it is therefore, decided to maintain the separate generic identity of the genus *Coleus*.

The genus *Coleus* , is estimated to have about 150 species (Willis, 1966) of which seven are represented in India. The four naturally occurring species are - *C. amboinicus* Lour. (*C. aromaticus* Benth.), *C. forskohlii.*, Briq. (*C. barbatus* Benth.), *C. spicatus* Benth. (*C. canisus* Roth) and an endemic *C. malabaricus* Benth. The three cultivar species are *C. vettiveroides* Jacob (*Plectranthus., tomentosus*), *C. scutellarioides* Benth. (*C. blumei* Benth.) an ornamental garden herb, and *C. rotundifolius* A. Chev. & Perr. (*C. tuberosus* Benth, *C. parviflorus* Benth.) which is grown for edible tubers in South India (Wealth of India, 1950). *C. forskohlii* , is the only naturally occurring species to have tuberous roots.

The specific epithet 'forskolaei' was assigned by Vahl in the year 1790 to Forsskal's specimen of *Plectranthus* in commemoration of Pehr Forsskal, a Swedish naturalist who died of malaria at Yerim in Arabia on July 11, 1763 (Friis, 1983) whilst on plant survey.

The correct binomial for the taxon is Coleus., *barbatus* (Andr.) Benth. Plectranthus., *barbatus* Andr., *P. forskohlaei* Ait, and *Coleus forskohlii* (Willd.) Briq., are the other commonly used synonyms (Bruce, 1935). The name *C. forskohlii* Briq. is preferred for this overview because of its consistent use in the earlier publications.

Recently a new species *Coleus grandis* Cramer, a tall, robust cultivar shrub growing in mesophytic conditions in Sri Lanka, resembling *C. forskohlii* in general appearance but differing in habit and habitat, was described by Cramer (1978). While reporting occurrence of this taxon in Africa as *C. barbatus* sensu Agnew (non Benth.), Willemse pointed out that the Cramer's species is conspecific with Plectranthus., *comosus* Sims (Willemse, 1985). However, the identity of the type specimen of Sims has been established as *C. barbatus* (Andr.) Benth. (Valdes *et al.*, 1987). *C. barbatus* sensu Agnew (*C. grandis* Cramer) collected from Kenya is chemically different from *C. forskohlii*, Briq. (*C. barbatus* Benth.) (Tandon *et al.*, 1979) supporting a species status to *C. grandis* Cramer.

1.2 Phytogeography and Ecology

C. forskohlii is a montane species occurring between the latitudes 31° N 30° S in palaeotrpic regions (Fig. 1). It occurs in central, east and south Africa, Sri Lanka, India, Nepal, Bhutan, and China. Occurrence of the taxon in Unnan province of south west China is reported by Prof. Jin-Qi-duan (personal communication). The plants of this species reported from Brazil are cultivars (Prof. Vaz, personal communication). Collections from these countries excluding China and Brazil are housed in Kew Herbarium (U.K.). Specimens procured by the author from India, Nepal and Brazil are deposited in the herbarium of Hoechst India Limited, Bombay. The species from Siam (Thailand) represented by a single collection in the U.S. National herbarium at the Smithsonian Institution, Washington DC, U.S.A. is communicated by the curators of the herbaria in Thailand to be unavailable. Occurrence of the maximum number of species of *Coleus* in Africa suggests Africa as the possible centre of origin for *Coleus*.

In India, *C. forskohlii* is observed to grow over a wide geographic range, between the latitudes 8° and 31°N, in subtropical and warm temperate climates of the Lesser Himalayas from Simla eastward to Bhutan, and on the hills of Central India, the Deccan plateau, Eastern plateau, rain-shadow regions of the Western and Eastern Hills (Mukherjee 1940), and the hills of North Gujarat (Fig. 2). Although altitudinal range for the Lesser Himalayas is between 1300-1685 m, the concentration of the species is more around 1450 m. in Kumaon Himalayas and adjoining north-west part of Nepal. In the peninsular India, the elevation belt is between

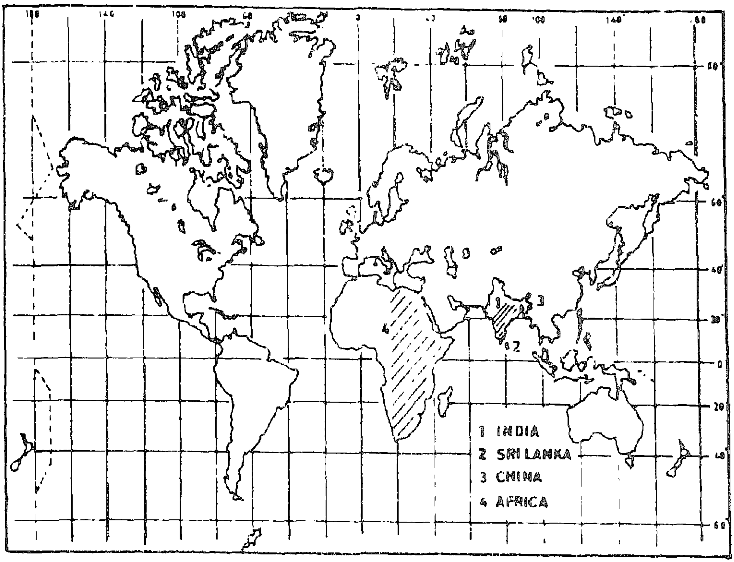

FIG. 1 GLOBAL DISTRIBUTION OF C. FORSKOHLII
(ZONES APPROXIMATE)

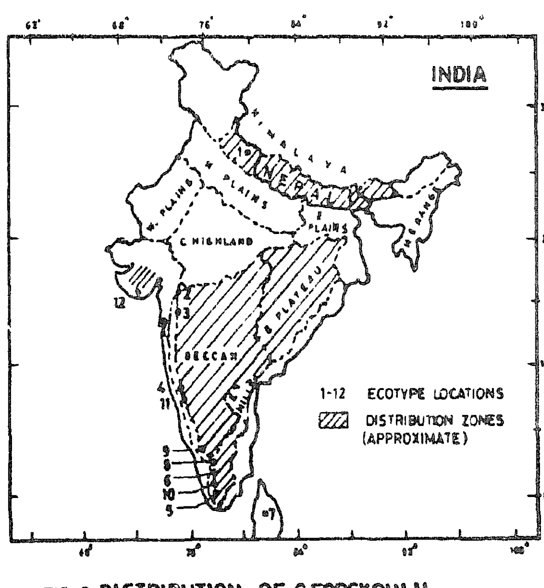

FIG. 2 DISTRIBUTION OF C. FORSKOHLII
AND LOCATIONS OF ECOTYPES

600-2000 m. Occurrence of the species though sporadic, is locally common (Shah, 1989).

The species prefers arid to semiarid habitat. It inhabits sun-exposed dry hill slopes, plateaus and shallow soil deposits of gorges, ridges, boulders, rocky and gravelly surfaces, walls, rock crevices, and occurs amidst short grasses and at periphery of scattered small bushes (Fig. 3). Soil profile of the habitat varies from 2.0 cm to 15.0 cm deep and is noticeably deficient in organic matter.

In order to understand the climatic and edaphic preferences of the species, climatic data and soil samples from 20 *Coleus* growing localities across India were analysed. Analysis of data indicates that these areas experience moderate annual rainfall of 400-1500 mm with intermittent dry period and a mean annual temperature of 18°- 27° C. Physical and chemical analysis of soil samples reveals that the species thrives well in sandy-loam or loamy soil with a pH range of 6.4 to 7.9. Specific requirement of the species, if any, could not be ascertained from the analysis.

1.3 Morphology

C. forskohlii growing in the Indian subcontinent is a herbaceous, pubescent, weakly aromatic species with annual stems and perennial rootstock. Roots are polymorphic - tuberous, semituberous and nontuberous (fibrous) (Fig. 4A). Tuberous roots are one to several, succulent but hard, tortuous or straight, short and stout or long and slender upto 56.0 cm in length. The cultivated types are fleshy and succulent, spindle-shaped or fusiform, generally long, not-so-stout, several and radially spread. Root tubers in both the kinds have white or orangish pink flesh, have bitter aftertaste, and are aromatic. Tuber skin is papery, yellowish brown, brownish or brownish-black depending upon colour of the soil substratum. The habit varies considerably from almost prostrate or shortly erect and decumbent to erect. Shoot height including inflorescence is 20.0-90.0 cm, sometimes reaching 120.0 cm in length. Stems arising from the root-stock are one to several, pubescent, sparsely or profusely branched. In some populations basal stems branch repeatedly and form a ground cover. Leaves are semisucculent, softly pubescent, ovate, obtuse and attenuate at base and crenate at margins. Lamina measures 6.0-13.0 cm in length and 3.5-6.0 cm in breadth. Bracts are imbricate, 4-ranked, compact, enclosing inflorescence at bud stage and drop off as the spike elongates. Flowers clustered in verticillasters, are borne on 8.0-55.0 cm long terminal racemes. Calyx is two-lipped with upper lobe broadly ovate and deflected when dry. Fruiting calyx is enlarged and has an annulus of white tufted wooly hairs within at throat. Corolla is generally bright blue, at times white or lilac and is characteristically bilabiate. Lower lip of the corolla is much longer, 1.6-2.5 cm long constricted at the middle and at the other end boat shaped. Stamens are four, didynamous, lying along the lower lip with exerted anthers. Filaments are connate at base forming a dorsally open tube enclosing the style Seeds (nutlets) are 3-4 per fruit, subspherical, shining black

when mature. Stems, under surface of leaves, bracts, calyx and corolla are all dotted with red glands. The basic morphology of the species, otherwise conforms to the characteristics of the family and the genus (Cooke, 1903).

1.4 Root Histology

Abraham *et al.*, (1988) observed pedicillate cytoplasmic vesicles containing yellowish to reddish-brown substance storing secondary metabolites in freehand sections of fibrous and tuberous roots of *C. forskohlii*, collected from Tamil Nadu and Kerala. The vesicles were observed to be common in cork cells, in vascular supply to the rootlet and at the periphery of the nematode infested tissue. Concentration of the vesicles around the infested zone suggests their protective role. The vesicles being exclusive to *C. forskohlii* assume diagnostic importance.

1.5 Intraspecific Variation

Morphological — In nature, *C. forskohlii* exhibits several fascinating morphological variations in the plants collected from different ecogeographic regions. Shah (1989) has given a comprehensive account of morphological, histological, cytological, palynological, phenological and agronomical variations in 10 natural and two cultivar ecotypes located at 12 diverse ecogeogrpahic sites (Fig. 2). Clones of diverse ecotypes (populations) were collected from their natural habitats and transplanted on a common habitat in Bombay under uniform cultural conditions Variations observed in these ecotypes were proved to be genetic in the transplant experiment. Striking morphological variations were observed in the roots, growth habit, shoot height, number of branches, pattern of branching, leaf morphology (size, shape and colour), floral bracts, inflorescence length, and indumentum. A few of these characters are illustrated in Figs. 3 and 4. Hegde (1992) evaluated the same 12 ecotypes under the Bangalore climatic conditions and grouped them in two clusters, one of fibrous rooted type with profuse branching and the second of tuberous rooted type with comparatively sparse branching with the exception of two of tuberous rooted type with profuse branching in metroglyph analysis. Vishwakarma *et al.*, (1988) reported varying root-yields ranging from 16.6 g to 203.3 g per plant based on data of 38 genotypes assembled from diverse sources and cultivating them under uniform cultural conditions. Bahl and Tyagi (1989) also refer to the existence of the different morphotypes in their germplasm stock. It is noteworthy to mention here that the plants with diverse root morphology and growth habits were observed by this author to be occurring in South India, especially Tamil Nadu and Kerala.

Forskolin variation — Existence of chemical races (chemotypes, chemical varieties) in the vegetable kingdom is well documented (Trease, 1985). *C. forskohlii* is replete with chemical races and both inter and intra-population variation in forskolin is reported. In the 38 collections from different locations, Vishwakarma *et al.*, (1988) reported variations in forskolin content from 0.01 to 0.44% in six

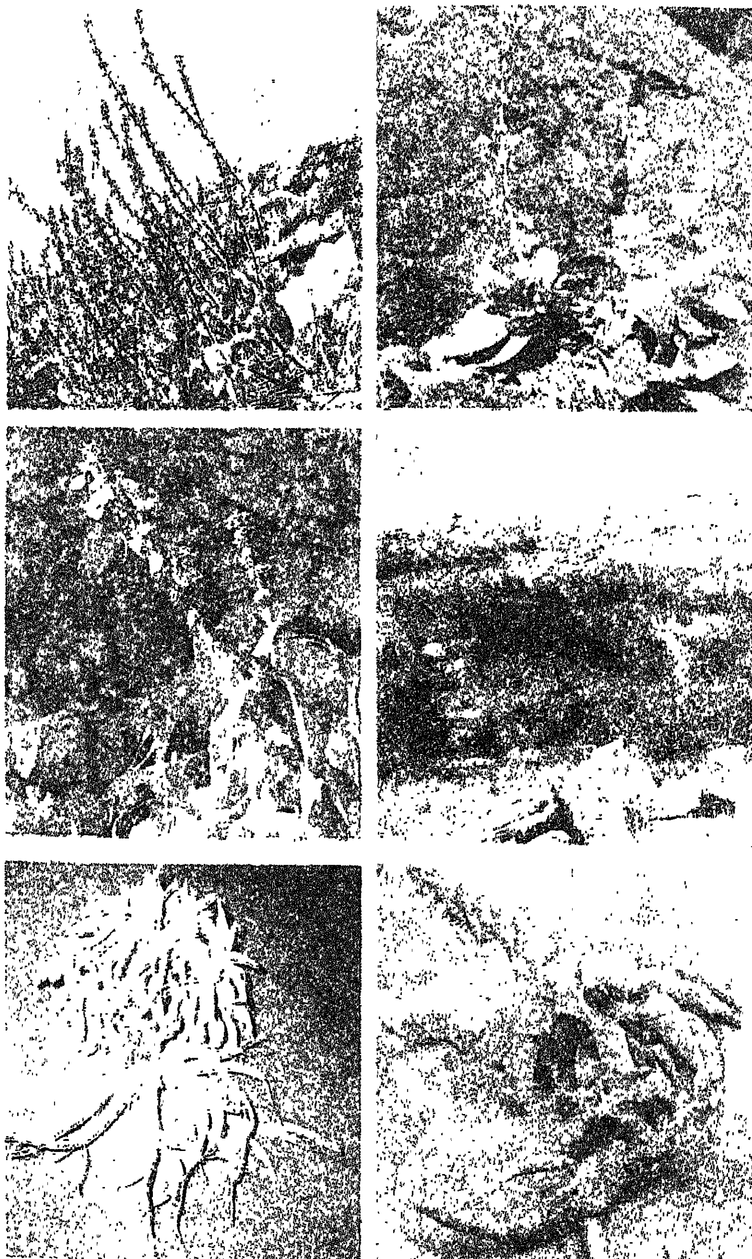

Fig. 3 *C. Forskohlii*: Habitat and Habit

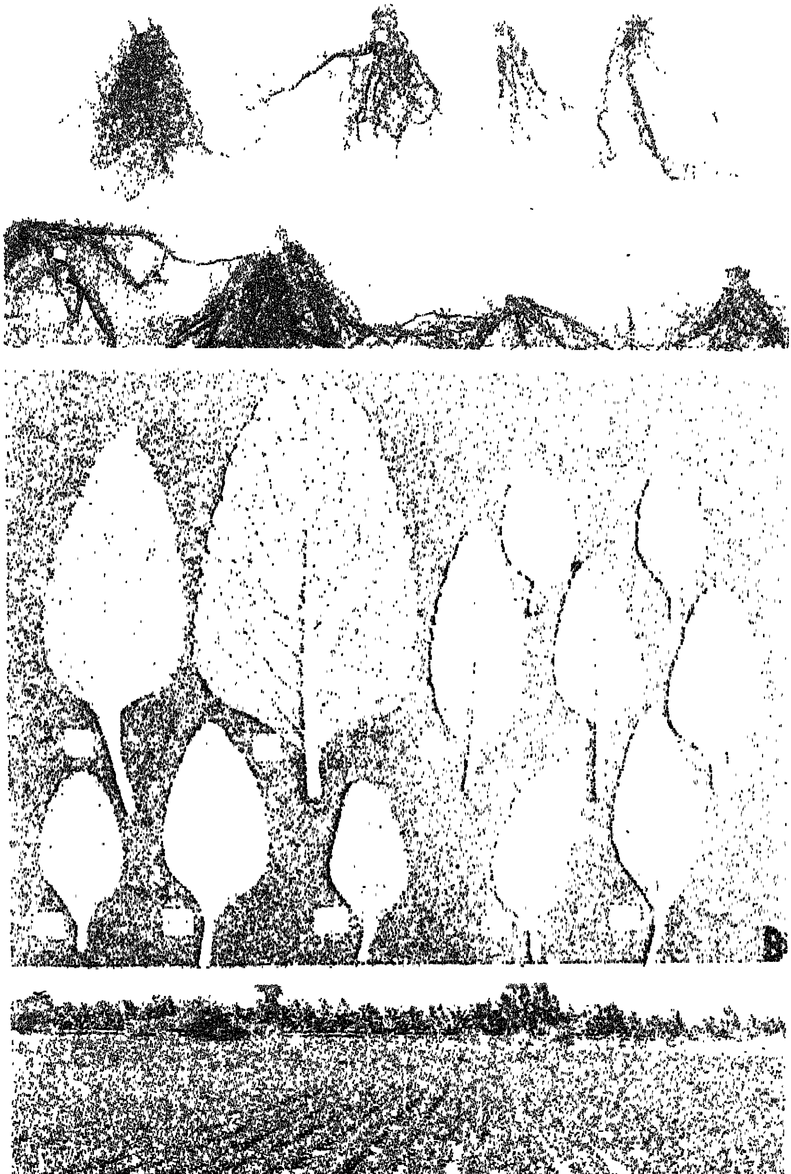

Fig. 4 A: Root Variation; B: Leaf Variation; C: Cultivation

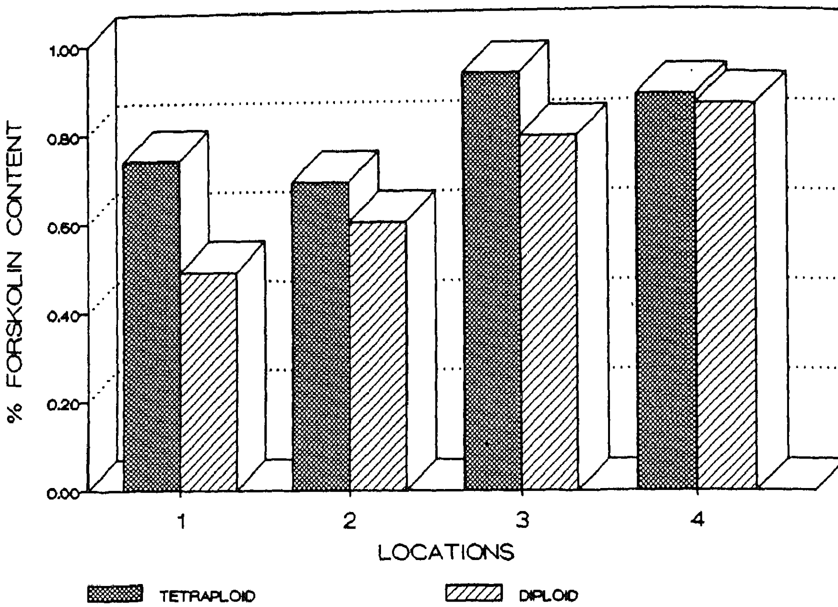

FIG. 5 EVALUATION OF TETRAPLOID vs DIPLOID MULTILOCATION FIELD TRIAL

months old dry roots. Scientists at Hoechst India Limited in their search for forskolin-rich strain, assayed 102 root samples of single clones and of populations collected from natural sources from different ecogeographic regions in India (70 from Kumaon and Garhwal Himalayas, 3 from Gujarat, 2 from Maharashtra, 8 from Karnataka, 13 from Tamil Nadu, 2 from Andhra Pradesh and 4 from Kerala) and more than 12 cultivar root samples from Gujarat, Maharashtra and Belgaum farms in Karnataka between the years 1982-1986 for forskolin content. Forskolin content in these samples ranged between 0.066% to 0.58% by dry weight. Differences for forskolin content in the root samples could not be correlated by this author with altitude, latitude, leaf morphology (size and colour of leaves), plant stature and habitat (grassy, gravelly, boulder, loose soil etc.). The elite clone selected from the above when grown under the climatic conditions of Bombay showed consistent forskolin content whereas when propagated in drier region of higher altitude at 900 m recorded higher forskolin yield of 0.7% to 0.8%.

1.6 Chemotaxonomy

C. forskohlii enjoys a unique position in the field of Chemotaxonomy in that it is the only species to contain forskolin. A chemotaxonomic oriented search (Shah *et al.*, 1980) for alternative and/or superior sources of forskolin within the family Labiatae in roots and shoots of six species belonging to the genus *Coleus* and 6 other species belonging to the allied genus Plectranthus all growing in India, indicated that only *C. forskohlii* contained forskolin. Chemical assay of roots and shoots of 24 species of the following genera (value in parenthesis refers to the number of species) viz. *Anisochilus*(4), *Hyptis* (1), *Lavandula* (1), *Basilicum* (syn. Moschosma) (1), *Ocimum* (1), *Orthosiphon* (3), *Anisomeles* (1), *Colebrookea* (1), *Dysophylla* (1), *Gomphostemma* (1), *Leucoceptrum* (1), *Leonurus* (1), *Nepeta* (1), *Pogostemon* (2), *Salvia* (3), and *Stachys* (1) in the family Labiatae also confirmed the exclusive presence of forskolin in *C. forskohlii* (unpublished data). Kanatani *et al.*, (1985) investigated 100 plant samples of medicinal and allied species belonging to 86 genera available in Japan for adenylate cyclase modulators. The samples included species of the genera like *Plectranthus*, *Coleus*, *Lamium*, *Pogostemon* etc. of the family Labiatae. *C. forskohlii* was procured from India as a reference sample.

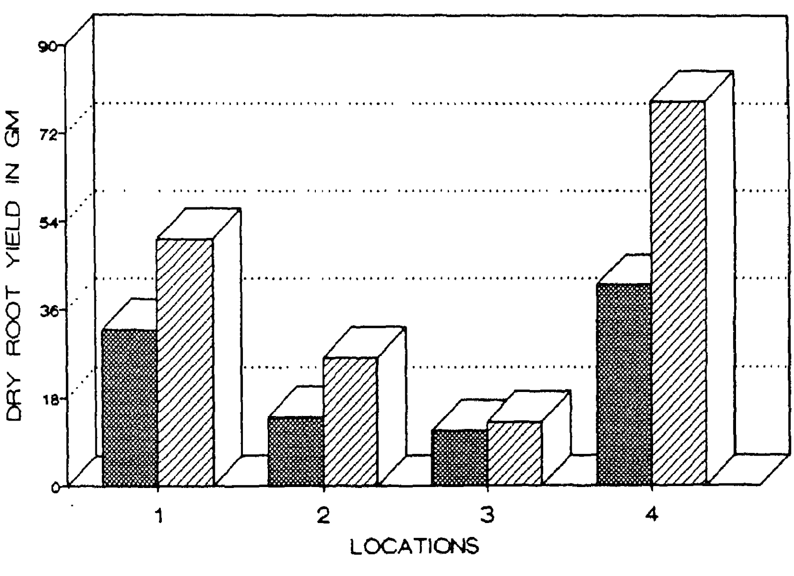

FIG. 6 EVALUATION OF TETRAPLOID vs DIPLOID MULTILOCATION FIELD TRIAL

None of the sample contained forskolin. Sevenet (1991) reported screening of several plant species including four medicinal Labiatae members *Leonurus cardiaca, Marrubium vulgare, Ballotta foetida* and *Glechoma hederacea* for cyclase modulating activity. None of the plant species exhibited forskolin-like activity.

C. forskohlii growing naturally in China is a chemical variant having 1-acetyl-forskoli as a major constituent (Prof. Jin-Qi-duan, 1990, communication to Dr. de Souza, then at Hoechst).

2. Cytogenetics

Collections of *C. forskohlii* from different places have shown varying chromosome numbers (Table 1). The variations in chromosomal counts were found for both tuberous as well as fibrous rooted varieties. Meiotic studies of five ecotypes (Shah, 1989) from geographically distant localities (Table 1) revealed complete pairing of chromosomes as bivalents at metaphase I in two ecotypes. In the other three ecotypes chromosomal associations at diakinesis consisted of trivalent (0-1), bivalents (4-8), univalents (10-15) and 2-5 loose pairs. Three bivalents were observed to be associated with nucleolus.

Table 1: Chromosome numbers of *C. forskohlii*

Chromosome number (2n)	Source Place	Author
28	Coimbatore(Tamil Nadu)	Reddy (1952)
	Saputara (Gujarat)	Shah (1989)
30	Bhimtal (Nainital, UP)	Mehra and Gill (1972)
	Bhimtal (Nainital, UP)	Shah (1989)
	Source not mentioned	Bahl and Tyagi (1988a)
	Maruthmalai (Tamil Nadu)	Shah (1989)
	Meenamparai (Kerala)	Shah (1989)
	Veraval cultivar (Gujarat)	Shah (1989)
	Kodaikanal (Tamil Nadu)	Bir and Saggoo (1985)
34	Pachmarhi (Central India)	Saggoo and Bir (1983)
34	South Africa	Riley and Hoff (1961) (Hegde, 1992)

Bahl and Tyagi (1988a) gave a detailed analysis of pachytene chromosomes in *C. forskohlii*, with a view to facilitate understanding of the evolutionary

relationship and preparing the linkage maps for cytogenetic and genetic research. Chromosome length varied between 15.00μ to 50-45μ and arm ratio between 0.09 to 0.90. Chromosome pairing at pachytene comprised 15 bivalents. Chromosomes 9 and 13 were associated with nucleolus. The same authors (1989) also reported variable amounts of nuclear DNA content in eight diploid collections drawn from different parts of India and in two induced autotetraploids. Autotetraploids yielded double the amount of DNA than diploid progenitors. Linear regression analysis of total chromosome length to the nuclear DNA amount showed a positive relationship.

According to Rife (1962) *C. forskohlii* , (2n=28) is an amphidiploid and has arisen from a cross between a species with the basic number of 8 and another with the basic number of 6 chromosomes. Basic number 15 (Mehra and Gill, 1972), and 16 and 17 (Bir and Saggoo, 1985) are also suggested. The latter authors are of the opinion that base number differences in various genera of the family could possibly be the result of aneuploidy at generic level.

3. Genetic Improvement

3.1 Polyploid Breeding

Genetic improvement through polyploid breeding and intraspecific intervarietal hybridization is being attempted with an objective to evolve a variety characterized by enhanced forskolin and root- mass yields. Dr. Krishnan and his students at Indian Institute of Horticultural Research (IIHR), Bangalore using twelve genotypes (ecotypes) provided by Hoechst India Limited, produced several autotetraploids (colchicine-treated shoot tips) and hybrids towards achieving of these objectives. Autotetraploids induced in six varieties - three tuberous rooted and three fibrous rooted varied in their morphological and physiological expression reflecting genotypic differences. Comparative evaluation of tetraploids vs diploids based on twenty five characters assessed quantitatively at 30, 60, 90, 135, 160, 190 and 230 days in three trials revealed that tetraploids were superior to their diploid progenitors in having thicker stems, broader and thicker leaves, taller shoots, larger size of stomata and of pollen grains whereas inferiority was observed in reduced number of primary branches and leaves per plant, short and compact inflorescence, poor pollen stainability, reduced number of stomata per unit area and initial slow growth (Hegde, 1992).

Autotetraploids CIMAP/KM produced from colchicine treated seeds (Bahl and Tyagi, 1988b) shared the features of initial slow growth, larger and thicker size of the leaves, increased size of stomata and pollen with the tetraploids developed

at Bangalore. However, CIMAP/KM differed from the Bangalore tetraploids in number of stomata per unit area. This difference could be attributed to their being different genotypes.

Autotetraploid of an elite variety developed at IIHR was evaluated for forskolin content and root yields at 180 days in a multilocational field trial at four sites, three in Tamil Nadu and one in Bangalore district, Karnataka. Percentage increase of forskolin in tetraploid over diploid in the trial varied from site to site being 2.3, 15, 17.7 and 51 (Fig. 5). Reduction in dry tuber yields of tetraploid at the respective site was 47.8, 46.1, 14 and 36.7 percent (Fig.6) (unpublished data). Despite enhancement of the forskolin content in tetraploid, slow growth and reduced root yields were major obstacles for its utilization.

3.2 Intervarietal Hybrids

Nine intervarietal hybrids developed by Hedge (1992) using the twelve varieties (six with tuberous roots, four fibrous rooted and two having semituberous roots) showed heterobeltiosis for several morphological and physiological features including roots. Promising hybrids AsxB and AsxD for root yields are under evaluation for forskolin content.

4. Cultivation

In the absence of developed packages of agropractices for cultivation of *C. forskohlii* , for medicinal purposes, the cultural practices followed for *C. forskohlii.*, raised for its roots (Garmar, Mainmul or Mangniberu) and/or tender shoots (Dala) in the states of Gujarat, Maharashtra and Karnataka is detailed below.

4.1 Gujarat and Maharashtra

Land preparation — *Coleus* needs a light sandy-loam or loamy-sandy soil which has been well manured in the previous crop season. *Coleus* is considered by farmers as an exhaustive crop and hence is never grown on the same land for successive years. Leguminous and other crops are grown for 3 to 4 or more years before the land is used once again for *Coleus* crop. The land is well prepared some weeks in advance by repeated ploughing in a criscross manner up to a depth of about 30 cm. Soil is divided into square plots of 1×1 m or 1.5×1.5 m when it is sandy as in Veraval or divided into ridges at 30 cm distance when it is non-sandy. It is very essential that soil is loose enough so as to allow growth and penetration of roots to their full length without hindrance.

Planting Material and Nursery — Terminal stem cuttings about 10 cm long with 3-4 pairs of leaves from rapidly growing shoots constitute the planting material. *Coleus* being a vegetatively propagated crop, for supply of the planting

material it is essential to maintain a nursery by planting terminal stem cuttings of the harvested crop under tree-shade in the months of May-June. To meet the requirements of planting material during planting season i.e. November-December, the plants from mother nursery are multiplied during August- September in nursery beds.

Cultural Practices — Field planting commences during the cool period soon after Deepawali, generally in the second fortnight of November and until about a month thereafter. The cuttings are planted on water drained ridges in a zigzag fashion on either side of the ridges at a distance of 15-20 cm between plants (Fig. 4C). Flood irrigation is provided initially everyday for the first three days after planting but subsequently frequency of irrigation is reduced to twice a week on establishment of the plants and until a month. Thereafter, depending upon water holding capacity of soil the irrigation frequency is restricted to once in 8-10 days interval. Thus 22-23 irrigations are given to the *Coleus* crop during its entire duration of 5-6 months.

Last irrigation is given 10-15 days before the scheduled harvest. Earthing up of the soil is carried out when the plants attain to a height of about 20-25 cm and weeding as and when necessary. Pinching of flower spikes as they appear is carried out until harvest. The root crop is ready for harvest after 5 months of planting.

The crop raised for tender shoots (dala) is given a top dressing with a pinch of ammonium sulphate after 15 days of planting to boost vegetative growth. The aerial parts are harvested after three months of planting.

Harvest and Marketing — *Coleus* crop is harvested 10-15 days after irrigation when the soil is more or less dry. The quality of tuberous roots for pickling purposes is not altered appreciably even when the crop is retained underground up to 6 months. This fact is exploited in the staggered harvesting of tubers to meet and maintain the market demand. As a result, the crop is harvested from April through early June, a season for pickle making.

Yield — The yield of fresh tubers per hectare ranges from 2.0 to 25.0 tonnes. The average yield, however, is about 12.5 to 15 tonnes/hectare.

4.2 Belgaum District in Karnataka

Coleus is raised as a rain-fed crop on the sugarcane and paddy field bunds. The bunds provide a most suitable substratum for tuber crop of *Coleus* since it is porous and rich in residual fertilizer. The planting period commences in "Magh Nakshatra" during the months of June-July. The plants are grown from the apical and internodal green stem cuttings with 3-4 nodes and internodes. Weeding is done as and when necessary. Irrigation provided to paddy and sugarcane fields irrigates the *Coleus* plants. *Coleus* grown on paddy field bunds needs one to two further irrigations after harvest of the paddy crop. Harvesting of tubers is done from the month of November through January.

4.3 Pests and Diseases

C. forskohlii crop is observed to be affected by root-gall disease caused by nematode, *Meloidogyne incognita*. Aerial parts of the nematode-infested plants though stunted appear as luxuriant as the normal plants. Root-yield of the infested plants though poor, its forskolin content is marginally higher (unpublished data).

Root-rot disease in the affected plants is manifested in the third month after planting by wilting of the leaves and turning of the basal stems brownish-black. Diseased roots at this stage are totally damaged. The disease is transmitted through soil irrigation. The causal organism is under identification. Aerial blight disease due to *Rhizoctonia solani* is reported for the species (Shukla *et al.*, 1993).

4.4 Optimisation of Agroparameters For New Agroclimatic Regions

For introducing *Coleus* into new agroclimatic regions and developing packages of agropractices for *Coleus* crop to improve root tuber yield/unit area, Hoechst India Limited initiated a collaborative project with the Tamil Nadu Agricultural University at Coimbatore. Adopting the agricultural practices followed in Gujarat, two tuberous rooted cultivar varieties V1 and V2 were used for determining adaptability of the variety, optimal spacing, standardizing nutritional requirement of nitrogen, phosphorus and potassium (NPK), and for optimal time of planting and harvest for Coimbatore environmental conditions. Variety V2 in replicated trials at 100 days was found to be more adaptive in plant establishment and root yields to Coimbatore conditions than the V1. Mean percentage of the established plants for V2 was 61.94 as against 11.39 of V1. Dry root tuber yield of V2 per plant at the end of five months of planting was 26.3 gm as against 13.1 gm of V1 (Veeraragavanthatham *et al.*, 1985).

Among the three spacings tried viz. 60×40 cm, 60×30 cm and 60×20cm, it was observed that the dry tuber yields/unit area was maximum in the closest spacing of 60×20 cm which accommodated maximum number of plants per unit area. Assuming 100% establishment, dry tuber yield after five months of planting (July 1985 - Nov. 1985) was estimated as 1.810, 2.020, and 2.270 t/ha for the three spacings, respectively (Veeraragavanthatham *et al.*, 1985; Seemanthini and Mohammed, 1989).

In the fertilizer trials laid in Randomised Block Design, 30 combinations of N, P, and K, using five levels of nitrogen - 0,40,80,100 and 160 kg/ha two levels of phosphorus - 0 and 60 kg/ha and three levels of potassium - 0,50 and 100 kg/ha were tried. A combination of 40 kg of nitrogen, 60 kg of phosphorus and 50 kg of potassium was assessed to be optimum for maximum fresh (33.120 t/ha) and dry (3.584 t/ha) tuber yields in the crop (Veeraragavanthatham *et al.*, 1988; Seemanthini and Mohammed, 1989).

In order to determine optimal time of planting and harvest, plantings were made at a spacing of 60×20 cm in the first week of every month for a year and plants

were harvested after 5, 5.5, 6 and 6.5 months of growth period. Months of June-July for planting and harvest after 5.5 months of growth period were determined to be optimal for maximum dry tuber yields under the Coimbatore conditions. Reduction in tuber yield was recorded when the crop was left unharvested beyond 5.5 months (unpublished data).

Manual weeding once in a month controlled the weeds better than using of pre-emergent weedicides viz. Fusilande, Goal, Delchlor and Basalin (unpublished data).

Between the two nematocides, Thimmet and Carbofuran, the latter @ 1 kg ai/ha was found to control the nematode.

4.5 Experimental Cultivation

Srivastava *et al.*, (1986) reported increased coleonol (forskolin) content in the plants sprayed with 150 ppm of indole acetic acid after 12 days of planting. Plants were harvested after four and six months of planting for determination of coleonol content. Path-coefficient analysis of the data revealed that the plants with less leaf area and high dry weight of roots yielded more coleonol (forskolin).

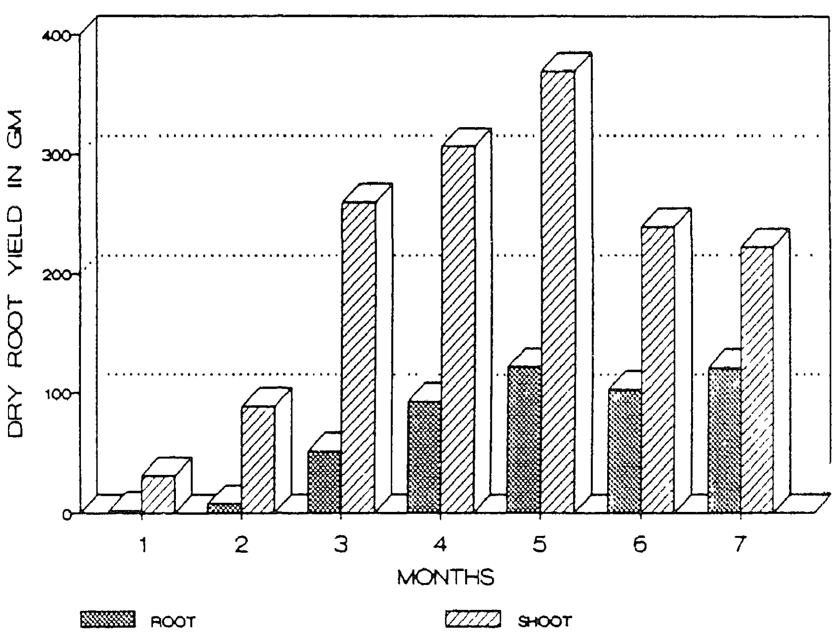

FIG. 7 GROWTH DYNAMICS OF ROOT AND SHOOT

Valdes *et al.*, (1987) reported 432 g yield of dried tubers for 60 plants raised from seeds and harvested after nine months of sowing as against 732 g of dried tubers for the same number of plants raised from stem cuttings. Plants raised from seeds provided 2g of forskolin.

Shah (1989) evaluated twelve varieties (ecotypes) over five years (1986-90) under the environmental conditions of Bombay and found variety 11 to be superior in tuber yields. Plantings made in the months of October, November and January provided better fresh root yield/plant being 122.0g, 80.0 g and 90.0 g respectively as against June planting which produced only 10.0 g of roots. Optimal stage of harvest was determined to be between 5 and 6 months after planting in the growth dynamic studies of root and shoot (Tables 2 and 3; Fig.7). Root-mass increased from the second month onward upto five months followed by a short reduction in the sixth month which was made up in the seventh month. Increase in root yields is statistically insignificant after five months. The reduction of root yield in sixth month is attributed to fall of basal leaves and survival of the plants on the food reserve of the roots during that period. Fresh foliage appearing subsequently help to building up of the reserve in the following month.

Table 2: Root yield in first seven months

Fresh Root	Months after planting						
Yield g/pl	1	2	3	4	5	6	7
a. Mean	2.2	8.1	51.4	93.5*	121.9	103.2**	121.0#
b. Range	2-3	3-20	36-73	56-164	33-224	86-151	29-248
c. n	5	9	8	10	9	5	5
d. SE	0.20	1.83	4.11	10.73	24.50	12.99	39.14

Planting Date : 21.10.87

*Significant at $P < 0.25$, **$P < 0.025$. # not significant

Table 3: Shoot yield in first seven months

Fresh Shoot	Months after planting						
Yield g/pl	1	2	3	4	5	6	7
a. Mean	31.0	88.88	260.1	306.3	369.5	239.2	222.4
b. Range	25.38	24.180	137-405	210-353	126-998	127- 328	73-480
c. n	5	9	8	10	9	5	5

4.6 Drying of the Root Tubers for Forskolin

Root tubers being succulent and with protective skin need to be finely sliced or cut vertically and then chopped, manually or shredded in a cutting machine, exposing maximum succulent surface for rapid drying. Natural drying by sunshine on a clean floor is practiced by the cultivators. Difference in forskolin content of the sun-dried and shade-dried root samples is insignificant (unpublished data).

5. Biotechnology

Plant cell, tissue and organ cultures can be alternative sources for forskolin and clones of *C. forskohlii*. Mersigner *et al.*, (1988) used a two step suspension culture for production of forskolin and 1,9-dideoxyforskolin. The growth media consisted of B5 medium supplemented with 0.5 mg/l of 2,4-D (2,4-dichlorophenoxyacetic acid) and 0.2 mg/l of kinetin. After six weeks the cells were transferred to a production medium containing IBA (Indolebutyric acid) which induced biosynthesis of secondary metabolites. The production of forskolin was 314 mg/kg dry cell weight. Forskolin production, however, was not stable over successive subculturing and the biosynthetic capacity of the culture was lost after 3-4 years of subculturing. Subsequently callus culture from another plant was used to standardise media and to study the other growth aspects. Forskolin production by cell culture was also studied as function of effect of light and plant growth regulator of different types in varying concentrations. From these studies it was concluded that IBA enhanced biosynthesis of forskolin. Light grown cultures produced 1000 mg/kg dry cell weight of forskolin as compared to dark cultures which yielded only 493 mg/kg dry cell weight. The morphology of calli was markedly affected by light in that they were hollow upto 4.5 cm diameter with hairy structure within while the dark calli showed some amount of differentiation in the form of roots. These cultures were scaled up in a 20 litre air lift bioreactor and 200 litre pilot reactor in dark. The yield of forskolin in the scaled up cultures was comparable to the amount present in plant roots.

The problem of unstable production of secondary metabolite faced in cell culture technique can be overcome to a certain extent by using differentiated organ cultures viz. roots which are known to be stable for biosynthesis of the secondary metabolites. Krombholz and coworkers (1992) initiated normal root cultures from primary callus which were derived from internodes. Initial studies were carried out in shake flasks and subsequently scaled up in a 20 litre bioreactor. The cultures were grown in dark at 25°C + 2°C. These normal root cultures were slow in growth and turned brown after 10-14 days. The roots when transferred to a fresh medium containing 1.0 ppm of IBA and 0.6 gm/1 of casein hydrolysate grew faster and branched profusely. The same research team also studied hairy root cultures or transformed root cultures by inoculating sterile young leaves with an overnight culture of *Agrobacterium rhizogenes* strain 15834. These roots grew much faster

than normal roots resulting in a high biomass and reducing the fermentation time. The main observations in the above studies were, (i) normal root cultures produced more forskolin in bioreactors than in the shake flasks, (ii) root growth and forskolin production was not affected by shear, (iii) transformed roots (hairy) showed the same secondary metabolite profile as normal roots, (iv) primary callus derived root cultures produced 127-1200 mg/kg dry mass of forskolin at the end of the cycle and 3.5 mg/l at the end of 35 days, and (v) the roots were sensitive to auxin concentration. For optimisation of forskolin production, the authors suggested media screening and other culture conditions along with the strain screening.

Multiple shoots were initiated from aseptic cotyledonous leaves and hypocotyle on a Murashige and Skoog's (MS) basal medium supplemented with 1 mg/l of BAP and 1 and 2 mg/l of NAA by Sen *et al.*, (1992). Forskolin content of roots of *in vitro* grown shoots was estimated to be $9\times10^{-3}\%$ on a dry weight basis. Thus micropropagation can be used as a tissue culture nursery for clonal propagation of elite varieties of *Coleus* in field and for forskolin production as well.

Biotransformation

Additional sources of forskolin can be achieved by biotransformation of the inactive metabolite 1,9-dideoxyforskolin also present in the same plant in appreciable quantity. Microbial hydroxylation carried out by the fungus *Scopulariopsis* could convert 7-deacetyl-1,9-dideoxyforskolin to 7-deacetyl-forskolin by introducing hydroxyl groups in 1- and 9- positions. The transformation product, 7-deacetyl forskolin is readily convertible chemically to forskolin by selective acetylation procedure.

6. Chemistry

Pharmacological screening of ethanol and dichloromethane extract of root of *C. forskohlii* displayed antihypertensive, spasmolytic and positive inotropic activities (Bhakuni *et al.*, 1971 de Souza, 1977). This observation led to activity-directed isolation of a labdane diterpenoid named Coleonol (Dubey *et al.*, 1974; Tandon *et al.*, 1977) and Forskolin (Bhat *et al.*, 1977). These two compounds differed, as

originally thought, in stereochemistry of the acetate group at position 7. X-ray crystallography, spectral data and chemical transformations have now established the identity of coleonol with forskolin (Fig.8) (Ramakumar *et al.*, 1985; Viswanathan and Gawad, 1985; Saksena *et al.*, 1985).

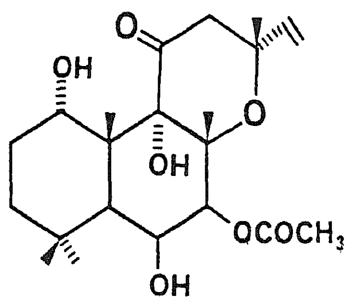

FIG.8 Forskolin

Forskolin, 7B-acetoxy-8, 13-epoxy-1, 6B,9-trihydroxylabd- 14en-11-one is one of the major diterpenoids present in the roots (< 0.1% to 0.58%) and shows poor solubility in water. Interesting structural features of forskolin molecule, its pharmacological profile and its unique adenylate cyclase stimulant activity attracted medicinal chemists to semisynthesise derivatives of forskolin with a view to improve its solubility in water and pharmacological profile. More than 150 derivatives of forskolin were prepared in order to understand structure-activity relationship. The derivatives were tested for cardiovascular activities and adenylate cyclase stimulant activity (Bhat *et al.*, 1983; Seasmon *et al.*, 1983; Laurenza *et al.*, 1987). Aminoacyl derivatives of forskolin were found to be highly active and soluble in water as well. One such aminoacyl derivative 6-(piperidinoacetyl)-7-deacetylforskolin showed positive inotropic activity more potent than forskolin (Khandelwal *et al.*, 1988). The unusual structural features of the forskolin molecule attracted organic chemists for its total synthesis. Research efforts describing strategies for synthesis and ultimate total synthesis of forskolin are reviewed by Bhat (1993).

Several secondary metabolites have been isolated from the root extract. 1,9-dideoxyforskolin, a major diterpenoid in the roots has anti-inflammatory property.

9-deoxyforskolin, a minor diterpenoid displayed weak forskolin-like activities (Bhat *et al.*, 1983). Epideoxycoleonol (epideoxyforskolin) occurring as a minor component displayed significant blood pressure lowering activity (Tandon *et al.*, 1992).

Species of *Coleus* growing in Africa and Brazil have provided abietane diterpenoids. Ruedi and Eugster (1972 and 1973) reported Coleon E and Coleon F from leaf glands of *C. barbatus* sensu Agnew (now a new species *C. grandis* Cramer), *C. kilimandscheri*, and Coleus., sp. collected from east Africa. Wang *et al.*, (1973, 1974) isolated barbatusin and cyclobutatusin from leaf extract of *C. barbatus* Benth. growing in Brazil. Barbatusol derived from the stem bark of *C. barbatus* Benth. exhibited hypotensive activity (Kelecom, 1983).

Information of phytochemical and biological activity of the roots of *C. forskohlii.*, (*C. barbatus* Benth.) growing outside India is unavailable. It is desirable that roots of *C. forskohlii* and allied species from the areas of their occurrence are also chemically screened for presence of forskolin. Until then, Indian *C. forskohlii* , will remain the sole source of forskolin.

7. Pharmacology and Therapeutic Potential

Forskolin, a labdane diterpenoid, isolated from the roots of *C. forskohlii* , exhibited unique activation of adenylate cyclase enzyme and thereby increased level of cyclic AMP in the mammalian cells (Metzger and Lindner, 1981; Seamon *et al.*, 1981). Unlike other known activators, forskolin acts directly at catalytic unit or at a protein closely related to it, requiring neither a membrane receptor nor the nucleotide-binding subunit of the enzyme system (Seamon and Daly, 1981a). The enzyme adenylate cyclase is present throughout the body in the membranes of cells. It is a key enzyme for modulation of various cellular physiological processes. This discovery was a milestone in the cellular physiology. Thus forskolin became a potent research tool for biomedical scientists to study the role of CAMP in regulation of physiological functions. The compound was introduced in market as a biochemical by Calbiochem Corporation, Lajola, California, U.S.A. Various biochemical, physiological and pharmacological properties reported for forskolin are summarised below.

Forskolin exhibited reversible adenylate cyclase stimulating activity in isolated cell membranes, intact cells and isolated organ systems (Metzger and Lindner, 1981; Seamon, 1984; Seamon and Daly, 1986). Forskolin potentiated effects of hormones on the adenylate cyclase system (Seamon *et al.*, 1981).

The physiological and the pharmacological responses evoked by forskolin are hypotensive, spasmolytic and positive inotropic (Lindner *et al.*, 1978; Dubey *et al.*, 1981), anti-inflammatory (Dohadwalla, 1986), antiplatelet aggregation (Siegel *et al.*, 1982), bronchodilator (Burka, 1983; Lichey *et al.*, 1984), antiglaucoma (Caprioli and Sears, 1983), enhanced acid and pepsinogen secretion by salivary glands and colon (Hersey *et al.*, 1983), antimetastatic (Agarwal and Parks, 1983), stimulation of nerve regeneration (Kilmer and Carlsen, 1984), stimulation of lipolysis of fat deposits, steroidogenesis (Ammon and Muller, 1985), and activation of proliferation of human epidermal keratinocytes in culture (Artuc *et al.*, 1988).

Forskolin has therapeutic potential in treatment of congestive cardiomyopathy, glaucoma, and bronchial asthma (de Souza *et al.*, 1983; Rupp *et al.*, 1986). The drug forskolin was well tolerated by patients treated for congestive cardiomyopathy and no major side effects were noted (Beaumann *et al.*, 1990).

Compound NKH 477, 6-(3-dimethylamino propionyl) forskolin hydrochloride, a water soluble derivative of forskolin is being developed by Nippon Kayaku in Japan for congestive cardiomyopathy. The compound has been tested for its efficacy with promising results in 68 patients with severe cardiac insufficiency in a multicentre study (Mamomura and Takano, 1993). 5-6-deoxy-7-deacetyl-7-methylaminocarbonyl forskolin (HIL 568), a forskolin derivative is a potent antiglaucoma agent (de Souza, 1993). Kaik (1986) observed that forskolin by itself or in combination with longer acting bronchodilators can be expected to be effective in patients suffering from chronic obstructive lung disease.

Forskolin has recently been shown to elicit CAMP independent effects through modulation of nicotinic acetylcholine receptor channel desensitization, modulation of voltage-gated potassium channels, reversal of multidrug resistance and inhibition of glucose transport protein (Laurenza *et al.*, 1989; Morris *et al.*, 1991). The reader is referred to the works cited for more details. The biochemical aspects, pharmacological profile, and clinical application of forskolin have been reviewed by Seamon and Daly (1981, 1986), Seamon (1984), Daly (1984), de Souza *et al.*, (1983), Ammon and Muller (1985), de Souza (1986), Rupp *et al.*, (1986), de Souza and Shah (1988), and Bhat (1993).

Phytotherapeutic Potential

Kansal *et al.*, (1978) studied effects of pulverised whole plant on 14 patients suffering from hypertension. Administration of 100-200 mg of plant powder t.d.s. brought blood pressure down to normal in a week's time in 13 patients.

Pulverised root (900 mg/kg body wt.), ethanol extract of root (900 mg/kg body wt.) and Coleonol A (300 mg/kg body wt.) showed some degree of amoebicidal activity against *Entamoeba histolytica* in rats (Varma *et al.*, 1990).

de Souza (1993), based on the antithrombotic activity of the standardised ethanol extract of "Makandi" (root of *C. forskohlii*) in rats proposed development of a phytotherapeutic preparation of Makandi as an antithrombotic agent.

Concluding Remarks

Discovery of forskolin as a unique research tool to study the role of CAMP in various physiological processes and its wide spectrum of biological activities with therapeutic importance to mankind prompted the study of *Coleus forskohlii* as a source plant of forskolin. Ecological adaptability of *C. forskohlii* to a variety of climatic and growing conditions in India has given rise to numerous local land forms and chemical races. These, in turn, have produced phenotypically diverse populations providing plant breeders with a gene pool to evolve a variety characterised by enhanced forskolin content and root tuber productivity. Efforts of the scientists engaged in plant breeding have met with some success in producing a forskolin-rich variety. It is also possible to obtain forskolin through biotechnological approaches such as micropropagation of the elite genotypes rich in forskolin, hairy roots organ cultures and cell cultures. All these avenues, however, in India may not be cost-competitive with the conventional vegetative propagation considering the ease with which *C. forskohlii* propagates vegetatively and its relatively short growth period of 5-6 months. The plant cultivation is perhaps the only economically viable source at present for forskolin.

Acknowledgement

I wish to thank Dr.W.Badziong and Dr.Bansi Lal for their support to this review paper. I am indebted to Mr.M.R.Almeida, Dr.R.Krishnan and Dr.P.D.Bhatt for going through technical and language aspect of the manuscript. I acknowledge with thanks assistance of my colleagues and friends within and outside of the Hoechst research in preparation of this overview.

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Chemistry of *Tinospora* Species

Rakesh Maurya

Regional Research Laboratory
Jammu 180 001, India

FOR the past few years we have been engaged in the chemical studies of Indian origin *Tinospora* species. The major objectives of these studies were the isolation and characterisation of compounds responsible for the claimed biological effects of the plant, search for the presence these bioactive compounds and study of biologically inactive but chemically and structurally interesting compounds present in these plants.

Tinospora genus (Menispermaceae) consists of deciduous woody climber, distributed throughout tropics of Asia, Africa and Australia. The following six species of *Tinospora* have been investigated.

1. *Tinospora cordifolia* Miers.
2. *Tinospora malabarica* Miers.(syn. *T. sinensis* Mierr)
3. *Tinospora crispa* Miers.(syn.*T tuberculata* Beumee, *T. rumphii* Miers.)
4. *Tinospora capillipes* Ganep.
5. *Tinospora dentata* Diels.
6. *Tinospora glabra* Mierrill.

The first three species occur in the country[1]. The aqueous extract of these plants are widely used in the traditional system of medicine for the treatment of jaundice, rheumatism, urinary diseases, intermittent fever, eye and liver ailments. It is mentioned in Ayurveda for its adaptogenic and immuno-modulatory activity in fighting infections[1-4] The aqueous and alcoholic extract of the plant significantly reduces the blood glucose, it also showed protective effects on carbon tetrachloride

induced hepatotoxicity[5]. The starch obtained from the plant is used as medicinal agent in chronic diarrhoea and dysentery[1]. In view of these biological activities, we took *Tinospora cordifolia* for chemical investigation and focussed our attention towards the isolation of compounds from aqueous extract of this plant. Activity guided isolation technique have resulted in the isolation of three new clerodanes tinosponone (**1**), tinocordioside (**3**) and cordioside (**4**) and novel immuno-stimulant phenyl propene glycosides cordifolioside-A (**31**) and cordifolioside-B (**32**) and new ecdysteroid cordiol (**37**).

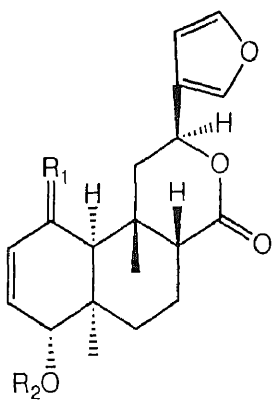
Despite structural complexity and interesting biological activities, no comprehensive review devoted specifically to *Tinospora* species is available to date. However, mention has been made to clerodane in general[7]. Approximately 50 compounds are presently known, a review devoted entirely to these is now desirable. The review covers the literature included up to March 1995 and comprehensive discussion on structure elucidation.

The results of chemical investigation of different Tinospora species are listed in Table 1 and the structures are shown in Chart 1 We have also tried to present The ^{1}H and ^{13}C NMR spectral data in Tables 2 and 3.

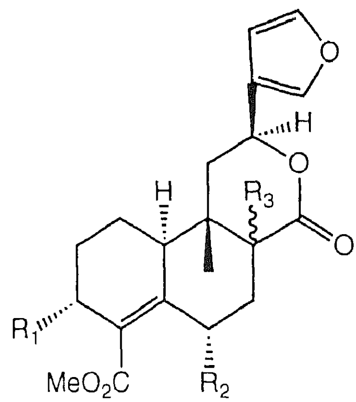
Tinospora species contains most important group of diterpene derivatives known as clerodane furano diterpene, which are responsible for bitterness[7,13]. The structural novelty and biological activity associated with these compounds attracted the attention of many chemists and biologists, resulting in the discovery of more than one hundred compounds of this group now known in the literature. Considerable structure elucidation work on these type of compounds have been done and today it is relatively easy to recognize clerodane furano diterpene from its diagnostic spectral features. A typical diterpenoid tinosponone[8] (**1**) of the clerodane series containing two tertiary methyls at C-9 and C-5. The characteristic ^{1}H and ^{13}C NMR signals (Tables 2 and 3) revealed the existence of a β-substituted furan ring (δH 6.38 s, 7.36 s and 7.42, δ C 108.41, 143.79, 139.66, and 125.09). The chemical shift and splitting pattern of proton at C-12 as double doublet at δ 5.57, J=12 4 Hz and two one-proton double doublets at δ2.35, J=15, 4 Hz and δ1.71, J=15, 12 Hz assigned to C-11eq and C-11ax protons are very characteristic and the signals for these hydrogens can be readily recognized. The hydrogens at C-8 and C-10 at δ 2.30 and 2.25 are also clearly observed. The presence of α, β-unsaturated ketone in ring-A was confirmed by doublet ascribed to H-2 at δ 5.97, J=10.8 Hz and double doublet to H-3 at δ 6.65, J=10.8 Hz. The β proton showed an additional coupling to a proton resonating at δ 4.35 assigned to the C-4 proton. The presence of carbinolic carbon was also evident from ^{13}C NMR signal at δ 70 57, this is also the site of glucosidic attachment as in tinosporaside[9] (**2**) The coupling constants for the methine protons H-1′ to H-5′ of hexose showed an all trans-axial relationship and together with the methylene H- 6′ resonances confirmed the identity of sugar as β-glucose. The acid hydrolysis of tinosporaside[8,9] (**2**) gave a hexose $[\alpha]_D + 48^{\circ}$,

Table 1: Chemical constituents of *Tinospora* species

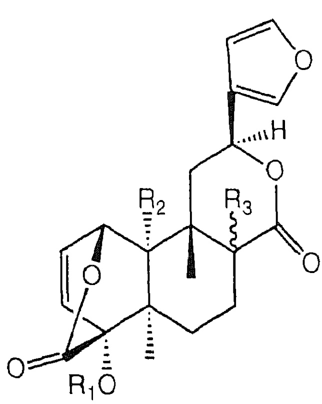
Structure No	Trivial name	Plant source	Plant part	References
1	Tinosponone	*T. cordifolia*	stem	8
2.	Tinosporaside	*T cordifolia*	stem	9
3	Tinocordioside	*T.cordifolia*	stem	8
4	Cordioside	*T.cordifolia*	stem	10
5.	-	*T.cordifolia*	stem	10, 11
6	Cordifoliside-A	*T cordifolia*	stem	12
7	Cordifoliside-C	*T.cordifolia*	stem	12
8	Cordifoliside-B	*T.cordifolia*	stem	12
9	Columbin	*T cordifolia*	stem	13
		T.dentata	tuberous	14
		T.capillipes	rhizome	15
10.	10 α-hydroxy columbin	*T malabarica*	stem	16
11	Isocolumbin	*T capillipes*	rhizome	13, 17
12	Tinoside	*T.cordifolia*	stem	13
13	Borapetol-A	*T.tuberculata*	stem	17
14.	Borapetoside-A	*T tuberculata*	stem	17
15	Borapetol-B	*T.tuberculata*	stem	18
16	Borapetoside-B	*T tuberculata*	stem	18
17	Borapetoside-C	*T tuberculata*	stem	19
	Tinocriposide	*T crispa*	stem	20
18	Borapetoside-G	*T.tuberculata*	stem	19
19.	Borapetoside-D	*T.tuberculata*	stem	19
20	Borapetoside-E	*T tuberculata*	stem	19
21	Borapetoside-F	*T tuberculata*	stem	19
22.	-	*T cordifolia*	stem	21
23	-	*T cordifolia*	stem	22
24.	-	*T.cordifolia*	stem	23
25	Tinotufolin-A	*T tuberculata*	leaves	24
26	Tinotufolin-B	*T tuberculata*	leaves	24
27.	Menispermacide	*T. malabarica*	stem	25
28.	Tinosporicide	*T.malabarica*	stem	26
29.	Malaborolide	*T.malabarica*	stem	27
30.	-	*T. cordifolia*	stem	28
31.	Cordifolioside- A	*T.cordifolia*	stem	29
32	Cordifolioside- B	*T.cordifolia*	stem	29
33.	-	*T cordifolia*	stem	30
34	Syringin	*T.cordifolia*	stem	30
35	-	*T.cordifolia*	stem	30
36	Sinapic acid	*T.cordifolia*	stem	31
37.	Cordiol	*T.cordifolia*	stem	32
38.	-	*T.cordifolia*	stem	33



1 R_1=O, R_2=H
2 R_1=O, R_2=Glu
2Ac R_1=O, R_2=Glu$(OAc)_4$
3 R_1=H, R_2=Glu
3Ac R_1=H, R_2=Glu$(OAC)_4$



4 R_1=H, R_2=OGlu, R_3=βOH
4Ac R_1=H, R_2=OGlu$(OAc)_4$, R_3=βOH
5 R_1=H, R_2=OGlu, R_3=αH
5Ac R_1=H, R_2=OGlu$(OAc)_4$, R_3=αH
6 R_1=OGlu, R_2=H, R_3=βH
7 R_1=H, R_2=OGlu, R_3=βH



9 R_1=R_2=H, R_3=βH
10 R_1=H, R_2=OH, R_3=βH
11 R_1=R_2=H, R_3=αH
12 R_1=Glu, R_2=H, R_3=αH

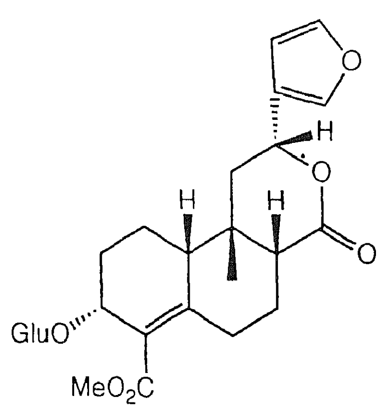


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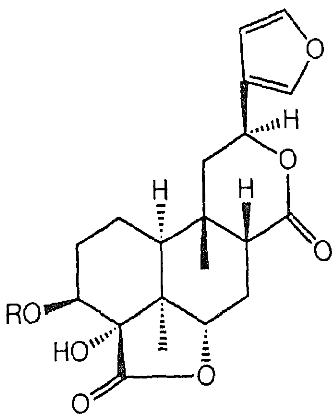
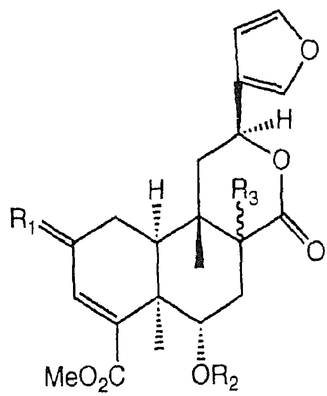
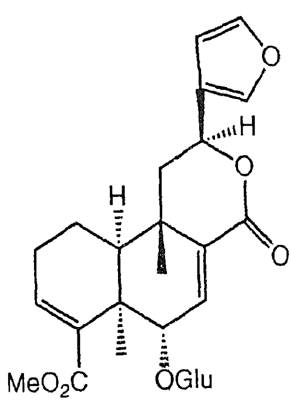
Chart 1: Chemical constituents of *Tinospora* Species



13 R=H
14 R=Glu



15 R_1=αOH, R_2=H, R_3=αH
16 R_1=αOH, R_2=Glu, R_3=αH
17 R_1=H, R_2=Glu, R_3=βH
18 R_1=O, R_2=Glu, R_3=αH



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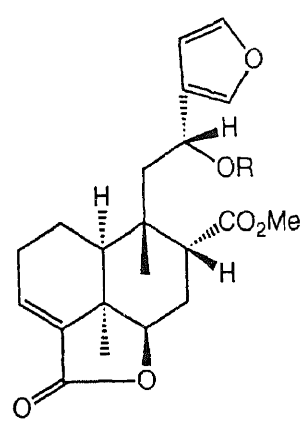
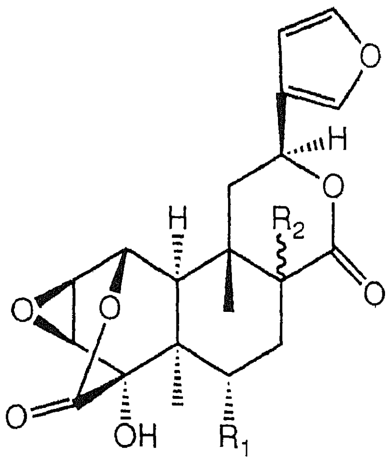
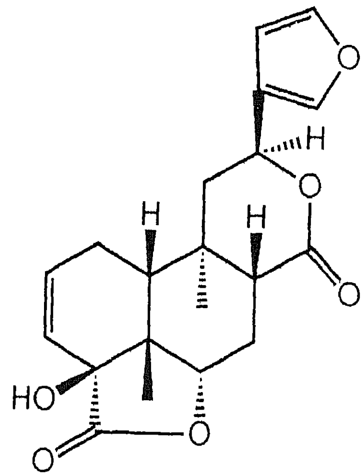


19 R=Glu(6-->1)Glu
20 R=Glu

Chart 1 Contd. : Chemical constituents of *Tinospora* Species



22 R_1=H, R_2=βH
23 R_1=OH, R_2=αH



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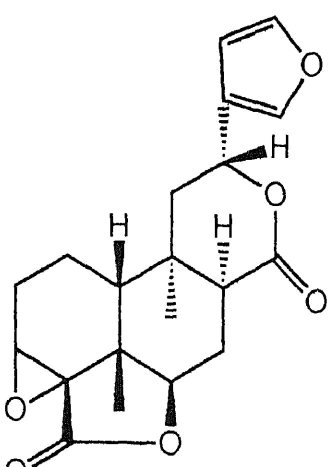

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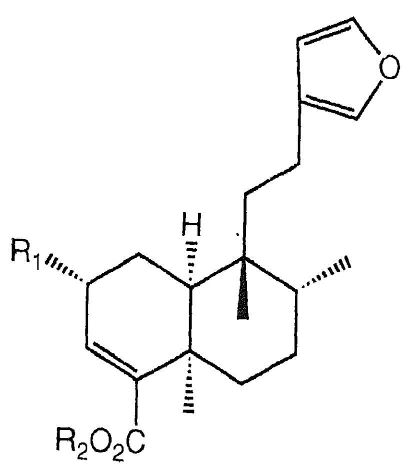
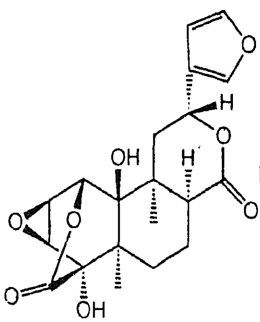


25 R_1=OH, R_2=Me
26 R_1=R_2=H

Chart 1 Contd. : Chemical constituents of *Tinospora* Species



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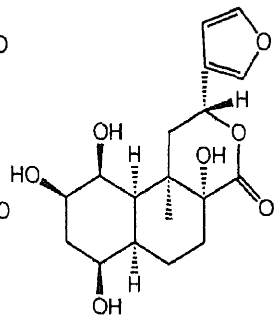

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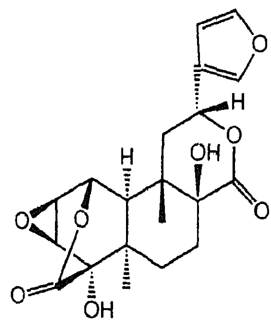

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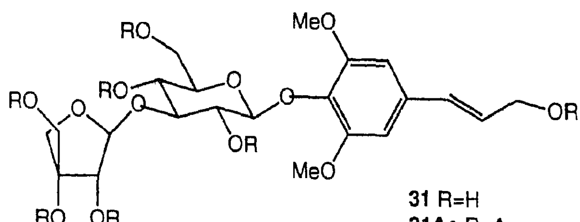
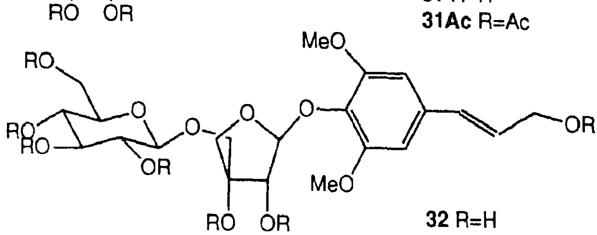


31 R=H
31Ac R=Ac



32 R=H
32Ac R=Ac

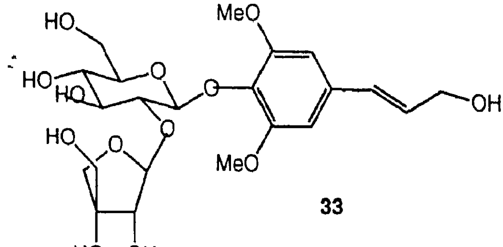


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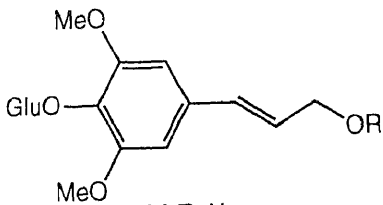
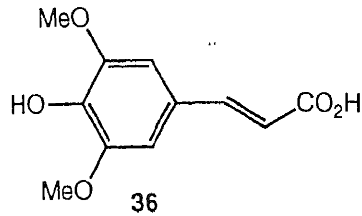
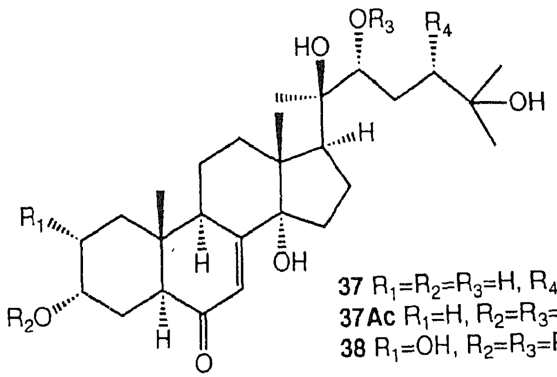
Chart 1 Continued : Chemical constituents of *Tinospora* Species



34 R=H
35 R=Me



36



37 $R_1=R_2=R_3=H$, $R_4=OH$
37Ac $R_1=H$, $R_2=R_3=Ac$, $R_4=OAc$
38 $R_1=OH$, $R_2=R_3=R_4=H$

Chart 1 Continued : Chemical constituents of *Tinospora* Species

thus establishing the occurrence of a β-D-glucose. The stereochemistry of compound **1** and **2** was explored through NOE experiments, confirming both A/B and B/C ring junctions *cis*.

The FAB mass of spectrum of tinosponone (**1**) and tinosporaside (**2**) showed characteristic ion peaks at m/z 369$[M+K]^+$, 353$[M+Na]^+$, 121, 95, 94, 81 and 531$[M+K]^+$, 515$[M+Na]^+$, 163, 121, 95, 94 and 81 respectively. The fragment at m/z 81 resulted from the cleavage of the C-11 and C-12 bond. The ion peak at m/z 94 and 95 were due to the cleavage of the δ-lactone ring along the C-9/C-11 and C-12 bond in ring-C. These observations clearly indicated that the furan ring occupied the C-12 position. The fragment at m/z 121 reveals that these compounds are furanoid diterpenes possessing an oxygen function at C-12 and an angular methyl group[20,34] at C-9. The fragment at m/z 163 in tinosporaside indicates that it contains sugar as hexose moiety.

The IR spectra of Tinosponone (**1**) indicates the presence of hydroxyls (3430 cm^{-1}), δ -lactone (1710 cm^{-1}), α,β-unsaturated ketone (1678 cm^{-1}) and furan (874 cm^{-1}) The UV absorption at 241 nm supported the presence of α,β-unsaturated ketone group.

The additional support for the tinosponone structure was confirmed by its transformation to tinosporaside tetraacetate[9] **2Ac**. Condensation of **1** with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric cyanide,

Table 2: ^{1}H NMR spectral data of compounds

Protonon	1[a]	2[d]	3Ac[d]
1			2.38 m
			1.59 m
2	5.97 d(10.8)	5.91 d (10.8)	6.48 m
3	6.65 dd(10.8, 5)	6.94 dd (10.8,5.2)	6.60 m
4	4.35 d (5)	4.38 dd (5.2)	4.22 d (5.2)
5			
6	2.25 m	1.99 brd (14)	2.20 m
	1.08 dt(14.4)	0.88 brt (15)	1.15 m
7	2.19 m	2.11 brd (14)	2.20 m
	1.65 dddd (14.2, 14, 8.2, 1.5)	1.50 brt (14)	1.59 m
8	2.30 brs	2.96 m	2.60 m
10	2.25 brs	2.45 s	1.30 m
11	2.35 dd (15, 4)	2.38 dd (15, 3.2)	2.28 dd (15, 3.6)
	1.71 dd (15, 12)	1.85 dd (15, 12.2)	1.88 dd (15, 2.2)
12	5.57 dd (12, 4)	5.57 dd (12.2, 3.2)	5.42 dd (12.2, 3.6)
14	6.38 brs	6.65 s	6.44 s
15	7.36 brs	7.68 s	7.44 s
16	7.42 s	7.72 s	7.49 s
18			
19	1.30 s	1.22 s	1.26 s
20	0.78 s	0.70 s	0.96 s
1′		4.33 d (7.8)	4.95 d(7.7)
2′		4.58 t (6)	5.05-5.31 m
3′		3.02 m	
4′		3.65 m	
5′		2.96 m	3.75 m
6′		3.12 m	4.30 dd (12.4, 4.48)
			4.16 dd (12.4, 2.8)
OH		5.25 d(6), 5.02d(6)	
		4.97 d(6), 3.45 brs	
OCOMe			2.15 s, 2.10 s,
			2.01 s, 2.0 s

Solvent for NMR a = $CDCl_3$, b=DMSO-D_6,c = Acetone-D_6 d = Methanol-D_4
Chemical shifts (δ) are expressed in parts per million from Me_4Si and coupling constants (J) in hertz. Multiplicity,s =singlet, d=doublet, dd=doublet of doublet, m=multiplet, br = broad, brs-broad singlet.

(Table 2 Contd.)

Table 2 Contd.: ^{1}H NMR Spectral Data of Compounds

Protonon	4Ac[a]	5[d]	6Ac[a]
1	1.49-1.95m	1.45 m	1.63 m
		1.54 m	1.95 m
2		1.56 m	1.50 m
		1.72 m	2.12 m
3		1.82 m	4.68 brs
4			
6	4.60 brs	4.49 brs	1 75 m
			2.37 m
7	2.97 m	2.64 brd (14 59)	1.83 m
	2.16 m	2.17 dd (14.5, 3.94)	3.16 d (11 1)
8		2.58 brs	2.42 brs
10	2.37 m	2.39 m	2.37 m
11	2.40 m	2.17 dd (14.5, 3.84)	1.8 m
	2.25 dd(16.6, 3.3)	1.94 dd (14.5, 12.3)	2.14 m
12	5.42 dd (12 6, 2.9)	5.57 dd (12.34, 3.32)	5.45 dd (12, 3.4)
14	6.48 s	6.65 brs	6.41 brs
15	7.43 s	7.68 brs	7.45 brs
16	7.50 s	7.71 brs	7.40 brs
18			
19			
20	0.97 s	0.92 s	0.96 s
CO_2Me	3.74 s	3.65 s	3.76 s
1′	4.61 d(8)	4.48 brs	4.64 d (7.43)
2′	5.18 t (9.6)	4.12 brd (7.8)	4 95 m
3′	4.9 t (9 8)	4.23 t (5.3)	5.12 t (9 5)
4′	5.07 t (9.7)	3.45 m	5.05 t (9.5)
5′	3.68 m	2.83 m	3.68 m
6′	4.27 dd (12.22, 4.3)	3.13 m	4.10 dd (12.2, 2)
	5 15 dd (12.22, 1.2)		4.21 dd (12.2, 5)
OCOMe	2.0 s, 2.01 s, 2.1 s		1.96 s, 1.97 s, 2.0 s, 2.07 s

(Table 2 Contd.)

Table 2 Contd.: ^{1}H NMR spectral data of compounds

Proton on	**7Ac**[a]	**8Ac**[a]	**9**[a]
1	1.56	1.45 m	5.45 dd (1.5, 5)
	1.84 m	1.58 m	
2	1 74 m	1 55 m	6.59 dd (5.8)
	2 49 m	1.78 m	
3	2.08 m	4 55 brs	6.34 dd (1.5, 8)
	2.82 m		
4			
5			
6	4.57 brs	2.15 m	1.69 dd (8, 14)
		3.1 brd (11)	
7	1 56 m	1.63 m	1.38 m
	1.86 m	1.98 m	
8	2 37 m	2.70 brd (11)	2.82 m
10	2.44 m	2.27 m	1.89 brs
11	1.91 m	1.86 m	2.47 dd (12, 4)
	2.25 m	2.17 m	2.55 m
12	5.45 brd (11.7)	5.38 dd(11, 5.5)	5 68 dd (12, 4)
14	6.42 brs	6.39 brs	6.66 d (1.5)
15	7.50 brs	7 43 brs	7 64 dd (1.5,,1)
16	7.38 brs	7.49 brs	7.68 d (1)
18			
19			1.20 s
20	1.01 s	0.86 s	0.95 s
CO_2Me	3 74 s	3.74 s	
1′	4 60 d (7.9)	4.5 d(7.7)	
2′	4.9 t (8 5)	4.87 m	
3′	5.18 t (9.5)	5.15 t (9.5)	
4′	5 28 t (9.5)	5.05 t (9.5)	
5′	3.66 m	3.62 m	
6′	4.06 d (12.2)	4.06 dd (12, 4.4)	
	4.12 dd (12.2, 4 25)	4.24 dd (12, 4.4)	
OCOMe	1.96 s, 1.99 s, 2.07 s	1.95 s, 1.97 s	
		1.98 s, 2.06 s	

(Table 2 Contd.)

Table 2 Contd.: [1] NMR spectral data of compounds

Proton on	**10**[a]	**13**[a]	**14**[b]	**15**[b]
1	5.4 dd (8,5)			
2	6.52 dd (7.8, 5)			
3	6.25 dd (7.8.,1.8).	4.04 m.	4.04 m.	6.34 m
4				
5				
6	1.17 m	5.02 m	5.02 m	5.02 m
7	1.17 m			
8		2.78 m	2.78 m	2.76 m
10				
11	2.45 dd (14.5, 11.5)			
	2 25 dd (14.5, 11.5)			
12	5.6 dd (11.5, 5)	5.9 dd(12, 4)	5.90 dd(12, 4)	5.49 dd(12, 4)
14	6.63 dd (1 7,1)	6 55 brs	6.55 brs	6.56 brs
15,	7 64 dd (1.7, 1.1)	7.51 brs	7.51 brs	7.65 brs
16	7.71 dd(1.17, 1)	7.64 brs	7 64 brs	7.71 brs
18				
19	1 21 s	1.23 s	1.23 s	1.45 s
20	1 07 s	1.12 s	1.12 s	0.85 s
CO_2Me				3 69 s
1′			4.48 d(7.99)	
2′			4.12 d(7.84)	
3′			4.23 t(5.3)	
4′			3.45 m	
6′			2.83 m	
			3.13 m	

(Table 2 Contd)

Table 2 Contd: ^{1}H NMR Spectral Data of Compounds

Protonon	16[b]	17[a]	19[b]	20[a]
1				
2				
3	6.34 m	6 79 dd(4.6, 3.6)	6.94 dd(4.1, 3 6)	7.01 dd(4.1, 3.6)
4				
6		4 63 brd (3.1)	5.25 brd(5.1)	5.46 brd(5.1)
7				
8	2 78	2.66 dd(13.2, 5.2)		2.71 brd(5.1)
10		2.22 m	1 85 m	2.0 m
11				
12	5.49 dd(12, 4)	5.5 dd(12 6, 11.7)	5.09 dd(8.2, 3 6)	5.24 dd(8.2, 3.6)
14	6 56 brs	6.47 m	6.5m	6.54 m
15	7.65 brs	7 41 m	7 58 m	7.36 m
16	7 71 brs	7 56 m	7 68 m	7.52 m
18				
19	1 41 s	1 39 s	1.23 s	1.32 s
20	0.85 s	1.08 s	1.23 s	1.27 s
CO_2Me		3.73 s	3.68 s	3 73 s
1′	4 48 d(7 99)	4 44 d(6.6)	4.21 d(6 6)	4.25 d(7.2)
2′	4 12 d(7 84)			
3′	4 23 t(5.3)			
4′	3 45 m			
5′	2.83			
6′	3 13 m			
1″			4 07 d(6.6)	

(*Table 2 Contd.*)

Table 2 Contd.: ^{1}H NMR Spectral Data of Compounds

Proton	21[a]	22[c]	23[c]	24[a]
1		4.99 d(2.8)	5.14 d(2.7)	1.74 m
2		3.93 dd(4.3, 2.9)	3.93 dd(4.3, 3)	6.41 m
3	6.63 dd(4.1, 3.1)	3.63 d(4.3)	3.66 d(4.5)	6.41 m
4				
6	4.63 d(4.1)	1.51 dd(14.3, 8.3)	2.99 m	5.15 dd(10.5, 2)
		1.91 m		
7		2.93 m	1.70 m	2.07 m
		1.91 m	1.64 m	2.64 m
8		2.40 m	1.55 m	2.41 dd(11, 2)
10	2.30 m	1.94 brs	2.16 brs	1.38 m
11		2.54 dd(14.3, 12.2)	2.41 dd(14.5, 11)	1.95 dd(14.4, 10.9)
		2.29 dd(14.3, 4)	2.27(14.5, 5.6)	2.20 dd (14.4, 4.5)
12	5.22 dd(10.2, 4.1)	5.67 dd(12.1, 4)	5.75 dd(12.2, 5.8)	5.4 dd (10.9, 4.5)
14	6.43 m	6.45 m	6.53 brs	6.32 dd(7.8, 1.6)
15	7.4 m	7.69 m	7.56 brs	7.48 m
16	7.48 m	7.74 m	7.59 brs	7.43 m
18				
19	1.39 s	1.10 s	1.29 s	1.25 s
20	1.41 s	1.04 s	1.21 s	1.06 s
OH			5.02 s, 4.91 s	3.88 s
CO_2Me	3.72 s			
1′	4.48 d(7.2)			

(Table 2 Contd.)

Table 2 Contd.: ^{1}H NMR Spectral Data of Compounds

Protonon	25[a]	26[a]	27[a]
1	2.01 ddd(15, 8.7, 6.3)	1.90 ddd(15.4, 10, 8.8)	1.54 m
	2.36 ddd(15, 8.7, 1.3)	2.05 dddd(15.4, 8.8, 8.8, 6.6)	1.73 m
2	4.57 ddd(8.7, 8.7, 3.5)	2.24 m	2.07 m
			2.20 m
3	6.36 d (3.5)	6.83 t (3.7)	3.20 m
4			
6	1.55 m	2.75 m	4.60 dd(13.1, 11.9)
	1.72 m	1.13 m	
7	1.17 m	1.18 m	2.40 m
	1.26 m	1.31 m	2.21 m
8	1.54 m	1.59 m	2.65 dd(13.1, 5.2)
10	1.71 dd(6.3, 1.3)	1.51 dd(6.6, 1)	1.90 dd(12.9, 5.4)
11	1.14 m	1.55 m	1.97 dd(12.6, 5.3)
	2.43 dd(11.3, 3.8)	1.72 ddd(13.9, 11.7, 5.1)	2.32 dd(15.3, 4.7)
12	1.02 m	2.31 m	5.64 dd(12.6, 4.7)
	2.30 m	2.31 m	
14	6.27 m	6.27 m	6.41 dd(1.8, 0.8)
15	7.22 m	7.22 m	7.42 t(1.8)
16	7.36 m	7.36 m	7.46 m
17	0.81 d(6.9)	0.81 d(5.9)	
19	1.40 s	1.26 s	1.21 s
20	0.76 s	0.80 s	1.13 s
CO_2Me	3.73 s		

(Table 2 Contd.)

Table 2 Contd.: ^{1}H NMR Spectral Data of Compounds

Proton on	**28**[b]	**29**[b]	**30**[b]
1	5.05 d(3)	3.51 m	5.05 d(1.9)
2	3.95 dd(4.4, 3)	3.79 m	3.96 dd(4.1, 1.9)
3	3.61 d(4.4)	1.57 dt(14.4, 3)	3.62 d(4.1)
		1.97 dt(14.4, 3.1)	
4		3.51 m	
5		1.40 m	
6	1.25 m	1.30 m	1.60 dd(14.8, 8.9)
	1.60 m	1.50 m	1.51 dd(14.4, 8.9)
7	1.42 m	1.50 m	1.34 m
	1.42 m	2.17 m.	
8	2.80 m		
10		1.91 t(10.2)	1.95 brs
11	2.25 dd(14.4, 11.6)	2.33 t(13)	2.25 dd(14.3, 12)
	2.05 dd(14.4, 5.1)	2.65 dd(13.4, 4)	2.08 dd(14.3, 5.1)
12	5.68 dd(11.6, 5.1)	5.69 dd(12, 4)	5.69 dd(11.8, 5.1)
14	6.53 dd(1.7, 0.8)	6.47 dd(1.7, 0.8)	6.55 brs
15	7.85 dd(1.7, 1.7)	7.63 t(1.7)	7.67 brs
16	7.71 dd(1.7, 0.8)	7.65 m	7.71 brs
19	1.08 s		1.11 s
20	1.08 s	1.03 s	1.06 s

(Table 2 Contd.)

Table 2 Contd.: ^{1}H NMR Spectral Data of Compounds

Proton on	**31Ac**[a]	**32Ac**[a]
1	4.71 d(6.5)	4.71 d(6.5)
2	6.21 dt(16.49, 6.5)	6.21 dt(16.49, 6.5)
3	6.56 d(16.49)	6.56 d(16.49)
2′	6.59 s	6.58 s
6′	6.59 s	6.58 s
OMe	3.82 s	3.83 s
Glucose		
1	4.97 d(7.8)	4.97 d(6.5)
2	5.26 m	5.27 m
3	3.75 t(7.82)	5.27 m
4	5.26 m	5.27 m
5	3 59m	3.59m
6	4.04 dd(12.22, 1.2)	4.17 dd(12.2, 1.2)
	4.18 dd(12.22, 4.3)	4.22 dd(12.2, 4.3)
Apiose		
1	5.07 brs	4.87 s
2	5.19 brs	5.12 s
4	4.53 d(13.04)	4.21 d(13.04)
	4.64 d(13.04)	4.30 d(13.04)
5	4.04 d(9.78)	3.78 d(9.78)
	4.19 d(9.78)	3.78 d(9.78)
OCOMe	2.0-2.1	2.0-2.1
	37[b]	**37 Ac**[a]
1, 2, 4, 9. 11, 12, 15 16, 17, 23	1.19-2.1 m	1.40-1.91 m
3	4.20 m	5.38 brs
5	2.30 m	2.38 m
7	5.62 brs	5.88 brs
9	3.30 m	3.14 m
18	0.75 s	0.85 s
19	0.82 s	1.03 s
21	1.10 s	1.22 s
22	3.82 m	4.80 d(10)
24	4.20 m	5.09 brd(9.83)
26	1.10 s	1.24 s
27	1.10 s	1.27 s
OH	4.50-4.82 br	4.60 br
OCOMe		2 02 s, 2.12 s, 2.13 s

(Table 2 Contd.)

Table 3: ^{13}C NMR Spectral Data of Compounds

Carbon	1[a]	2[d]	3Ac[a]	4Ac[a]	5[d]	6Ac[a]
1	202.46	202.71	26.06	17.67	18.52	17.0
2	129.04	125.39	132.39	28.34	22.74	28.0
3	143.42	143.90	130.68	26.74	26.98	72.8
4	70.57	70.02	72.28	143.39	144.49	152.6
5	43.58	42.73	38.44	128.86	131.17	126.9
6	29.6	28.20	29.63	72.10	75.21	22.3
7	19.14	18.77	17.25	30.64	28.78	28.3
8	49.16	47.85	47.31	72.03	49.78	49.2
9	36.08	35.35	35.21	34.62	38.68	38.7
10	50.61	47.34	44.34	39.10	40.00	41.8
11	40.76	40.10	41.81	33.86	40.04	40.0
12	64.49	72.42	70.59	71.70	71.15	70.8
13	125.00	127.18	125.00	125.00	126.49	124.9
14	108.41	109.01	108.30	108.59	109.08	108.5
15	143.79	145.39	143.90	139.90	144.83	139.5
16	139.66	140.26	139.58	143.39	145.57	144.7
17	171.60	170.00	173.31	171.12	171.94	171.7
18	-	-	-	170.31	169.45	169.0
19	25.57	24.26	24.01	-	-	-
20	31.92	31.12	28.24	23.10	23.32	22.5
CO_2Me				51.48	51.48	51.6
1′		104.57	98.03	99.44	102.40	101.1
2′		73.49	71.81	71.10	72.36	72.8
3′		69.97	72.61	72.88	78.56	72.7
4′		69.83	68.50	68.43	72.14	68.6
5′		76.59	72.94	71.70	78.43	71.2
6′		61.16	62.03	62.43	63.45	71.2
O*CO*Me			170.75	170.20		170.5
			170.27	170.00		170.2
			169.37	169.90		169.3
			168.37	168.70		168.8
OCO*Me*			20.72	20.54		20.5
			20.70			
			20.62			
			20.55			

Solvent for NMR: a = $CDCl_3$, b=DMSO-D_6, c=Acetone-D_6, d=Methanol-D_4.

(Table 3 Contd.)

Table 3 Contd.: ^{13}C NMR Spectral Data of Compounds

Carbon	**7Ac**[a]	**8Ac**[a]	**9**[a]	**10**[a]	**13**[a]	**14**[b]
1	17.7	18.1	70.3	74.6	17.4	17.4
2	22.2	26.6	130.3	131.1	24.6	24.6
3	28.17	73.6	135.7	147.4	75.6	75.6
4	145.1	145.0	80.4	81.5	79.5	79.5
5	128.8	128.9	36.8	37.2	45.2	45.2
6	73.0	30.0	25.7	27.5	69.8	69.8
7	26.5	21.9	17.0	17.4	27.2	27.2
8	49.6	47.2	45.8	44.8	46.9	46.3
9	38.5	39.0	34.6	35.6	34.3	34.3
10	39 3	47.2	43.4	71.8	46.1	46.1
11	40.0	43.0	40.3	40.7	42.9	42.9
12	70.1	70.5	73.3	71.0	69.4	69.4
13	124.9	124.0	125.2	125.5	124.2	124.2
14	108.4	108.5	109.1	109.7	108.7	109.2
15	139.5	139.6	140.3	140.8	140.3	140.2
16	143.7	147 7	143.7	143.7	143.5	143.7
17	171.2	173.4	174.6	173.5	172.6	172.6
18	168.0	168.4	173 6	172.7	177.6	177.6
19	-	-	23.7	27.5	32.6	32 6
20	23.7	21.4	27 1	24.8	17.9	17.9
CO_2Me	51.4	51.5				
1′	99.3	99.6				102.4
2′	71 5	71.7				74.1
3′	72.9	72.9				76.7
4′	68.3	68.0				73.1
5′	71.6	71 6				75.8
6′	62.0	62.0				60.7
O*C*OMe	170.6	170.5				
	170 2	170.2				
	169.3	169.3				
	168.8	168.9				
OCO*Me*	20.5	20 6				

(Table 3 Contd.)

Table 3 Contd.: ^{13}C NMR Spectral Data of Compounds

Carbon	**15**[b]	**16**[b]	**17**[a]	**19**[b]	**20**[a]	**21**[a]
1	28.0	28.0	24.0	23.6	24.3	24.6
2	62.2	62.2	16.7	15.9	16.6	19.7
3	140.2	140.2	141.1	141.9	142.5	137.6
4	137.4	137.4	135.9	133.7	134.2	135.9
5	40.9	40.9	39.9	38.7	39.3	41.3
6	79.0	79.0	76.7	81.9	83 1	80.0
7	26.4	26.4	27.7	28.6	29.7	135.9
8	49.2	49.2	45.8	46.4	46.6	138.5
9	36.5	36.5	35.2	38.7	39.3	37.1
10	40.4	40.9	27.3	45.1	45.6	46.8
11	44.0	44.0	42.6	46.2	46.6	45.3
12	69.9	69.9	68.4	67.7	69.1	70.9
13	124.5	124.5	125.2	126.9	126.1	123.9
14	109.2	109.0	108.6	109.4	109.4	108.7
15	140.2	140.2	143.5	142.9	143.5	143.6
16	143.7	143.7	140.1	140.0	140.4	139.9
17	174.6	174.6	175.3	166.3	166.9	170.1
18	167.5	167.5	167.3	177.1	178.8	169.5
19	28.4	28.4	36.6	26.8	27.2	27.5
20	22.8	22.8	28.6	20.3	21.5	23.5
CO_2*Me*		51.6	51.6	51.4	51.7	52.3
1′		104.6	103.0	99.5	99.2	104.7
2′		73.9	73.7	73.6	73.7	74.1
3′		77.4	75.6	76.7	76.4	76.4
4′		69.4	69.9	70.7	70.4	69.9
5′		76.6	75.0	75.6	75.6	75.6
6′		61.0	60.9	69.0	62.0	61.9
1"				103.4		
2"				73.6		
3"				76.7		
4"				70.7		
5"				76.7		
6"				61.3		

(Table 3 Contd.)

Table 3 Contd.: ^{13}C NMR Spectral Data of Compounds

Carbon	**22**[c]	**23**[c]	**24**[a]	**25**[a]	**26**[a]	**27**[a]
1	71.2	71.5	73.8	29.0	16.9	19.6
2	50.1	50.5	130.6	65.1	24.5	27.4
3	51.6	51.8	137.0	137.4	142.4	57.5
4	81.5	79 1	80.3	142.3	137.8	81.7
5	35.8	40.2	35.2	39.8	40.3	46.9
6	26.6	73.8	74.1	38.3	36.8	75.8
7	17 1	27.6	25.5	28.4	28.7	25.5
8	44 2	27 2	47.7	37.4	37.9	46.9
9	41.7	41 7	37.1	37.0	36.3	35.9
10	46 4	48 3	44 4	49.1	45 5	47 5
11	41 4	37.4	41.9	36.9	38.5	44.2
12	71 6	71 7	70.6	18.0	18.1	70 7
13	126 6	127 2	124 8	125 5	125 7	124 2
14	109 5	109 6	108 3	110 0	111.0	108.3
15	140 7	140 7	139 6	138 5	138 4	143.9
16	144 4	144.7	143.9	142 8	142.7	139.7
17	173 8	177 0	173.3	16.1	16.0	172.1
18	172 2	173.0	168.8	168.7	173.7	177.6
19	28 1	23 4	28.3	34.8	33.4	23.6
20	20.1	21.4	24.3	19.1	18.0	20.1
CO_2*Me*				51 7		

(Table 3 Contd.)

Table 3 Contd: ^{13}C NMR Spectral Data of Compounds

Carbon	**28**[b]	**29**[b]	**30**[b]
1	70.0	72.5	69.9
2	51.1	72.0	49.2
3	49.2	35.5	51.1
4	80.4	67.3	80.4
5	40.6	39.0	38.9
6	26.2	25.6	26.1
7	26.6	29.4	26.6
8	45.8	74.7	71.7
9	38.7	39.2	40.5
10	·71.8	35.8	45.6
11	34.9	35.0	34 5
12	70.5	71.0	70.6
13	125.6	126.0	125.5
14	108.9	108.9	109.1
15	139.9	143.4	140.1
16	143.8	139.7	143.9
17	171.4	171.8	173.1
18	172.9	-	171.6
19	22.7	-	22.8
20	20.1	15.1	20.1

(Table 3 Contd.)

Table 3 Contd.: ^{13}C NMR Spectral Data of Compounds

Carbon	**31Ac**[a]	**32Ac**[a]
1	64.9	64.9
2	123.1	123.1
3	134.0	134.0
1′	132.9	132.0
2′	104.2	104.2
3′	153.0	153.1
4′	133.0	133.0
5′	153.0	153.1
6′	104.2	104.2
OMe	56.4	56.4
Glucose		
1	101.1	101.1
2	72.0	72.0
3	72.9	72.0
4	69.0	69.0
5	72.7	73.1
6	62.5	62.6
Apiose		
1	107.5	107.6
2	81.8	81.8
3	83.5	78.7
4	76.5	74.3
5	62.8	67.0
OC*O*Me	169.0-172.0	169.0-170.0
OCO*Me*	20.5	20.1-20.3

(Table 3 Contd.)

Table 3 Contd.: ^{13}C NMR Spectral Data of Compounds

Carbon	**37**[b]	**37Ac**[a]
1	31.5	31.5
2	29.5	29.5
3	75.6	69.7
4	34.0	34.0
5	50.7	50.9
6	201.0	201.0
7	121 6	121.6
8	165.2	164.4
9	33.6	33.6
10	38.3	38.3
11	20.5	20.5
12	40.2	40.2
13	47.5	47.5
14	84.2	84.5
15	31 9	31.1
16	24.1	24.8
17	49.5	49.5
18	17.4	17.4
19	23.7	23.7
20	79.7	70 5
21	28.7	28.7
22	72.4	68.6
23	20.5	20.2
24	76.4	67.0
25	79.8	77.0
26	30.3	30.0
27	23.6	29.2
O*C*OMe		170.1
		170.2
		172.4
OCO*Me*		21.0
		21.1
		21.1
		29.2

gave the expected tinosporaside tetraacetate **2Ac**. The spectral properties of the synthetic product are identical with that of natural compound[9]. Thus tinosponone (**1**) is an aglycone of **2** presumed to have the same absolute stereochemistry.

The molecular formula of tinocordioside[8] (**3**) was as $C_{25}H_{34}O_9$ by FAB mass measurements, m/z 517[M+K]$^+$ and 501[M+Na]$^+$ and from the ^{13}C NMR spectra. The presence of four hydroxyls in the compound did not allow the resolution of all the protons in the ^{1}H NMR spectrum. Therefore, it was acetylated to yield tetraacetate derivative (**3Ac**) which has a comparable ^{1}H and ^{13}C NMR spectra to that of tinosporaside tetraacetate **2Ac**. The most striking difference in the spectrum is the lack of keto function at C-1 instead appearance of methylene protons as multiplets a δ H 1.59 and 2.38 (δ C 26.06) was observed. The two olefinic protons of ring-A resonated as multiplets at δ H 6.48, 6.40 and δ C 132.39, 130.68 assigned to C-2 and C-3 respectively. The proton at C-10 appeared as multiplet at 1.3 ppm. The signals of the other protons differed slightly from those of tinosporaside tetraacetate. Based on all these data we deduced a new furano diterpene glucoside structure **3**.

Cordioside[10] (**4**) afforded ions at 561[M+Na]$^+$ and 539[M+H]$^+$ in the FAB mass spectrum, suggesting the molecular formula $C_{26}H_{34}O_{12}$. The presence of hydroxyls in the compound did not allow the resolution of all the protons in the NMR spectrum. Therefore, it was converted to tetraacetate **4Ac**, $C_{34}H_{42}O_{16}$, 730[M+Na+H]$^+$. The FAB mass showed two major ion peaks at m/z 347 and 331 for the loss of hexose tetraacetate as an O-glycoside with retention and loss of ether oxygen. The identity of the sugar as β-D-glucose was confirmed by hydrolysis. The proton and carbon NMR spectra (Tables 2 and 3) together with a DEPT experiment indicated the presence of δ-lactone and ester carbonyls 171.12, 170.31 ppm, furan ring (δ H 6.48 s, 7.43 s, 7.50 s and δ C 108.59, 139.90, 143.39 and 125.0) ester methyl (δ 3.74 s, δ C 51.48), tetra-substituted olefin (δ C 143.39 and 128.86). An isolated ABX system at 5.42 [(dd, J=12.6, 2.9 Hz, 2.25 (dd, J=16.6, 3.3 Hz)] and a multiplet at 2.40 could be assigned to H-12 and H-11 respectively, with H-12 being axial. A broad singlet at δ 4.60 was assigned to proton at C-6 whereas multiplets appeared at 2.16 and 2.97 for the two protons at C-7. The ^{13}C NMR spectra of cordioside tetraacetate and **5Ac**[11] showed the close similarity but with a remarkable down field shift at the carbon atom bearing an additional hydroxyl group **4Ac** a double doublet at δ 49.46 in **5Ac** and a singlet at δ 72.03 in **4Ac** in relative ^{1}H couple spectra. By the comparison of the ^{1}H NMR spectra of **4Ac** and **5Ac**, we observed the absence of a multiplet at δ 2.44 in **4Ac** assigned to the C-8 proton in **5Ac** and instead the appearance of D_2O exchangeable singlet at δ 5.03 for a tertiary hydrox group, thus confirming its position. Cordioside (**4**) thus have the same stereochemistry as that of **5** and β-hydroxy group at the C-8 position. The NOE difference measurements were carried at different centres to determine the relative stereochemistry. The key observations were irradiation of the C-19 proton resulted in 5%

NOE of C-8OH, 2% NOE of C-11H and 4% NOE of C-6H while irradiation of C-6H resulted in 35 NOE of the C-19 proton, 4% NOE of C-7H, 2% NOE of C-8OH and 1% NOE of C-1'H, thereby establishing that C-6H, C-8OH, C-19 proton and C-11Ha were β-oriented, which confirmed the stereochemistry of cordioside.

The second major important class of compounds that have been isolated from *Tinospora* species are phenyl propene glycosides[29,30,35], such as cordifolioside-A (**31**) and cordifolioside-B (**32**). These two compounds are responsible for the immuno- modulator activity[29] in the *Tinospora cordifolia*. The FAB mass of cordifolioside-A(**31**) and cordifolioside-B (**32**) showed molecular weight of 504, corresponding to $C_{22}H_{32}O_{13}$, but the fragmentation pattern of the two differed. The proton and the carbon NMR spectra (Tables 2 and 3) of both were comparable indicating that the compounds were closely related. Cordifolioside-A and cordifolioside-B on acetylation gave heptaacetate **31Ac** and **32Ac** respectively. Cordifolioside-A heptaacetate in its proton spectrum (Table 2) showed a singlet for two aromatic proton at 6.59 ppm, two aromatic methoxyls at 3.85 ppm, trans olefinic protons as doublet at 6.56, J=16.49 Hz and as double of triplet at 6.21, J=16.49, 6.56 Hz and primary alcohol protons at 4.71 as doublet, J=6.5 Hz were characteristic of sinapyl alcohol[36] for the aglycon. The two sugars must be disaccharide's as confirmed by appearance of anomeric signal as doublet at δ 4.97, J=7.8 Hz, δ C 101.19 and as broad singlet at δ 5.07, δ C 107.53. Decoupling and coupling constant studies revealed that sugars were β-D-glucose and β-D-apiose. Characteristic fragment ions resulting from cleavage of interglycosidic linkage at m/z 547 for hexa-O-acetyl diglycosyl [api-glu(O Ac)$_6$]$^+$ at m/z 289 for tri-O-acetyl glucose [glu (OAc) $_3$]$^+$ and at m/z 259 for terminal tri-O-acetyl apiose [apio (OAc)$_3$ and confirmed the sequence of sugar as apiose-glucose which was linked directly to 3',5'-dimethoxyphenyl 2-*trans* propene-1-ol. A triplet at d 3.75, J=7.8 Hz assigned to C-3" proton of glucose unit showed no down field shift on acetylation **31→31Ac** indicated the site of linkage of apiose to glucose. The fact was further supported and confirmed by ^{1}H-^{1}H HOMO COSY spectrum of **31Ac**.

Cordifolioside-B heptaacetate (**32Ac**), $C_{36}H_{46}O_{20}$, corresponding to ion peak at m/z 837 [M+K]$^+$, 821 [M+Na]$^+$ in FAB mass spectrum. The ^{1}H and ^{13}C NMR spectra confirmed the sinapyl alcohol as the aglycone and β-D-glucose and β-D-apiose as sugar moieties. Close examination of ^{1}H and ^{13}C NMR spectra of two compounds indicated the difference in appearance of signals of sugar protons suggesting different interglycosidic linkage in cordifolioside-B heptaacetate (**32Ac**) as we observed protons of C- 5″ (apiose) unit appearing up field doublets at δ 3.78 and 3.87, J = 13.04 Hz and 2‴, 3‴, 4‴ protons of glucose unit appearing as multiplet at δ 5.27. This fact was confirmed by characteristic fragment ion resulting from cleavage of interglycosidic linkage in FAB mass spectrum showing major fragment at m/z 547 for hexa-O-acetyl diglycosyl [glu- api(OAc)$_6$]$^+$, at m/z 331 for terminal tetra-O-acetylglucose [glu(OAc)$_4$]$^+$ and 216 for di-O-acety-

lapiose[api(OAc)$_2$]$^+$. All these data confirmed the sequence of sugar as glucose-apiose (1‴→5") which was linked to 3′,5′-dimethyoxyphenyl 2-*trans* propene-1-ol.

Cordiol[32] (**37**) and **38**[33] is a new ecdysteroid isolated from most polar fraction of *T. cordifolia*. The IR of cordiol showed absorption for hydroxyl groups at 3480 and for α,β-unsaturated ketone at 1656 cm^{-1}. The presence of later functional group was further confirmed by the absorption maximum at 240 nm in UV spectrum. The ^{1}H, ^{13}C NMR, of both cordiol and its acetate **37Ac** (Tables 2 and 3), FAB mass spectra and colour test indicated that cordiol (**37**) possesses steroidal skeleton. The ion peak at m/z 481[M+H]$^+$ in FAB mass spectrum established the molecular formula for cordiol as $C_{27}H_{44}O_7$. ^{1}H NMR spectrum displayed the signals for two angular methyls at δ 0.75 and 0.82, three tertiary methyls at δ 2.20, multiplets of 18 protons in the region of δ1.19-2.10, a broad hump of 9 protons at δ 3.40-4.50 corresponding to hydroxy groups and *CH*-OH and a broad singlet at δ 5.62 characteristic of proton of α,β-unsaturated ketone in the molecule, ^{13}C NMR spectra (Table 3) showed the presence of 27 carbons in the molecule with characteristic absorption for α,β-unsaturated carbonyl at δ C 201.08, 121.60 and 165.23 and six oxygen bearing carbons at δ C 68.0, 84.2, 79.8, 72.4, 76.4 and 79.7.The mass spectrum showed peak at m/z 481[M+H]$^+$ together with major fragment ions at m/z 462, 445 and 427 corresponding to sequential loss of one, two and three molecule of water, m/z 347, 133 due to fragment between C-20 and C-22 and at m/z 303, 177 due to fission between C-17 to C-20. Similar fragmentation pattern was also observed in cordiol triacetate. This fragmentation is possible only if side chain has two hydroxyls at C-22 and C-24 supported by proton NMR of its acetate as broad doublet at δ 4.84, J=10 Hz and broad doublet at δ 5.09, J=9.83 Hz respectively. Further irradiations at δ 4.84 and 5.09 showed the change in the region of δ 1.4-1.91 indicating that they were separated by one carbon atom thus confirming their positions. A literature search of ecdysteroid from *T. cordifolia*[33] revealed the presence of tertiary hydroxyl at C-14 and secondary hydroxyl in ring-A with α,β-unsaturated carbonyl in ring-B and secondary hydroxyl group at C-3 in ring-A having α-orientation. The combination of all these spectral data led us to assign cordiol as 3α, 14α, 20β, 22α, 24α, 25-hexahydroxy choles-7-ene-6-one, a new natural product.

Besides the above discussed most important compounds, few less common flavanoids[37], lignan[39] and alkaloids[40-43] for *Tinospora* species have been isolated.

Acknowledgements

The author is grateful to Dr.R.S.Kapil, Director, Regional Research Laboratory, Jammu, for the support and encouragement in writing this review. Dr.Aruna Kapil

is thanked for help in bioassay. Dr. Versha Wazir and Anjulika Tyagi are thanked for the skillful efforts in isolating the compounds included in this review.

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Yew (*Taxus* spp.) — A New Look on Utilization, Cultivation and Conservation

Y.S.Bedi*, R.K.Ogra* Kiran Kaul*

Regional Research Laboratory
Extension Centre, Palampur* - 176 061

and

B.L.Kaul & R.S.Kapil

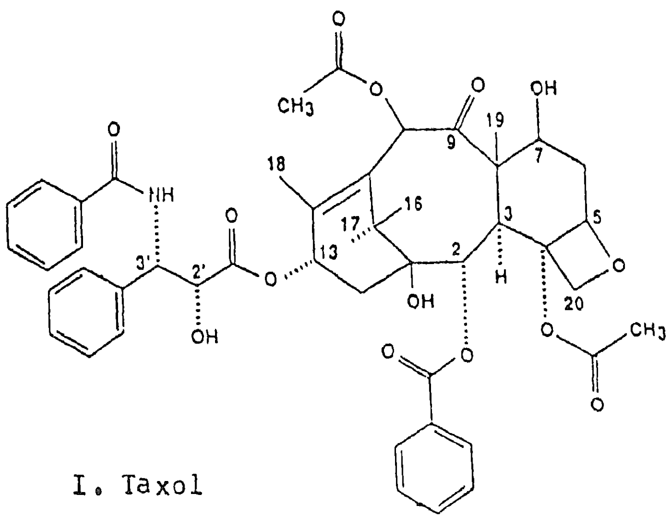
Regional Research Laboratory, Jammu - 180 001

Introduction

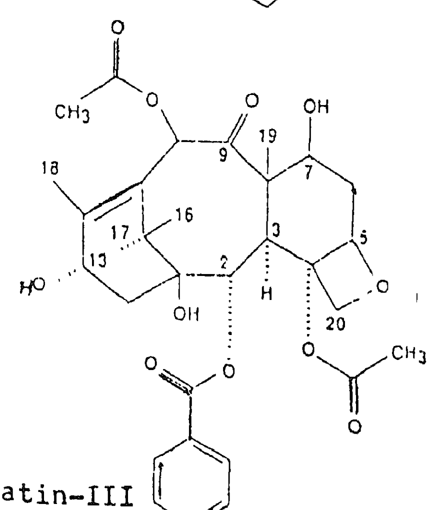
YEW, locally known in India as 'Barhmi', 'Rakhala', 'Thuner', has been used in herbal medicine for centuries. The traditional interaction of *Taxus* species with mankind has been varied in different parts of the world. However, it has been much known for childhood and animal poisoning (Smith 1989, Lucas 1856). Except for the red aril of the seeds, all parts of the plant are toxic. The bark was used for treatment of rheumatism, liver and urinary tract affections (Anonymous 1990). In Western Himalayas, a dilute decoction of the bark is taken during winters by nomadic people to increase body resistance to extreme cold. Due to highly tensile properties its wood was used to make bows and implements (Hartzel, 1991). Its wood has high calorific value and was used to ignite kilns to make a specific pottery in Kashmir. In Europe, yew is valued for making excellent hedges (Voliotis, 1986). The dried leaves are used in 'Havan', the Hindu sacrificial rites and incense materials.

The recent interest in screening plant based drug sources based on ethno-biology and bioassay directed analysis, has thrown *Taxus* as potent source of a new

group of diterpenoid compound grouped together as taxanes. Taxol (Fig. 1.1), first isolated from *T. brevifolia*, by Wani *et al.*, 1971 amongst this group has opened an entirely new approach in treating cancers by its effect on microtubule assembly in dividing cells. The taxanes are now known to be present in most of the *Taxus* spp. growing around the world (Beattie, 1992). The Himalayan yew *Taxus wallichiana* also contains taxanes in appreciable quantities. It has attracted attention particularly on account of high levels of baccatin-III (Fig.1.II), a precursor of taxanes in its leaves.



I. Taxol



II. Beccatin-III

Though other varieties of *Taxus* growing in America and Europe have been worked upon in detail with respect of silviculture, ecology, site requirements, reproductive biology, regeneration and genetics, negligible information is available

on the Himalayan yew. This article addresses to some of the recent developments in *Taxus* biology and need for conservation of Himalayan yew.

Current Importance

The current importance is mainly due to the demonstration of taxol on murine leukaemia system (Jacrot *et al.*, 1983). Later it was found to be effective against lung cancer, breast and colon cancers and other leukaemias (Suffness and Cordell, 1985), ovarian cancers (McGuire *et al.*, 1989). The structure activity relationships have been described in detail by Kingston *et al.*, (1990), and Guenard *et al.*, (1993). The C-13 acyl side chain, oxetane rings, a free 2-hydroxyl group, 3-amino group and absolute configuration at C_2 and C_3 contribute to the activity of taxanes to microtubule assay (Kingston *et al.*, 1990). Besides anticancer activity certain fractions from *Taxus* show antifungal activity (Young *et al.*, 1992) and insecticidal and metamorphosis disrupting effects on *Epilachea varivestris* (Schanne, 1988). While screening plants for silk enhancers in our laboratory, extracts from *Taxus* caused incomplete metamorphosis into pupa, when applied topically to 5th instar larvae.

Botany and Distribution

Genus *Taxus* belongs to taxales (related to coniferales) and is distributed around the globe in the north temperate areas of world. The plants usually exist in the understorey of mixed temperate forests as small evergreen trees and large shrubs, with a dense branch system with dark green long and narrow shiny leaves. Plants are dioecious. Fruits are solitary consisting a single dark ovoid seed surrounded by a red fleshy covering called the aril.

Flowering occurs in March-April. Many species and varieties exist, distinguished on the basis of bud scales, colour of leaves, leaf apices. However, finer distinctions are based on(i)number of stomate rows (ii) papillosity (iii)shape of epidermal cells (iv) curvature of lower midrib and stomatal regions (Sp jut 1992). The main species of interest are:

T. baccata (European yew)

T. cuspidata (Japanese yew)

T. chinensis (Chinese yew)

T. brevifolia (Western or Pacific yew)

T. wallichiana (Himalayan yew)

T. canadensis (Canadian yew)

A large number of horticultural yews are also available. *T. media* cv. 'Hicksii' is a hybrid of *T. baccata* and *T. cuspidata* and *T. x hunnewelliana* is a hybrid of

T. cuspidata and *T.canadensis*. Recently an electrophoretic characterization of *Taxus* cultivars has been conducted by Greer *et al.*, (1993).

The Himalayan yew *T. wallichiana* was earlier grouped with *T. baccata* (Raizada and Sahni 1960). It is found at an elevation of 7,000 to 11,000 ft. (Fig. 2) above m.s.l., however it is found at lower altitudes also in North Eastern hills of Himalayas. The variety growing in Western Himalayas (Himachal and J&K) is somewhat distinct from one growing in North East (Arunachal Pradesh, Meghalaya, Darjeeling, etc.). The former is more akin to *T. baccata* and the latter is more closer to *T. chinensis* in morphological characters. Cephalomannine, a diterpenoid isolated from a plant originally identified as *Cephalotaxus manni* (Powel *et al.*, 1974) was later shown to be *T. wallichiana* (Miller *et al.*, 1981). Difference also exists in the concentration of Taxane components between the two races (Kapil *et al.*, - unpublished data). Natural populations of Himalayan yew are found in mixed fir and cedrus forests in association with oaks and rhododendrons as understorey scattered plants. Usually the plants are found in the northern steep ridges.

Need for Conservation

The emergence of taxanes as new generation anticancer agents has resulted in sudden demand and supply crisis. Although the primary source of taxol has been the bark of *T. brevifolia*, it was later found (Vidensek *et al.*, 1990) that foliage of most of the species also contain extractable quantities of taxol and related compounds. The drug is present in miniscule quantities in the plant tissues. About 2.5 g. of this is required to treat an ovarian or breast cancer patient and conservative projections of the world demand are 700 kg/year. The present production level of taxol is about 350 kg due to non-availability of raw material. In order to produce 1 kg of taxol about 7,272 kg of bark is required and the amount of leaf clippings required is multifold. Consequently a drug supply crisis has emerged, resulting in blatant exploitation of *Taxus* flora. The Himalayan yew being no exception.

Management Strategies

Regional Research Laboratory, Jammu initiated a programme for conservation of the Himalayan yew on the following lines:

(a) Survey of *Taxus* growing area and status of regeneration in such pockets.

(b) Standardisation of macropropagation techniques and

(c) Devise a sustainable raw material system without affecting the natural habitat of *Taxus*.

Our surveys in Himachal Pradesh and parts of Northeast revealed that the plants are restricted to steep, mostly inacessible slopes on the northern ridges of mountains. The plants are heavily debarked and lopped; and most of the damage is

DISTRIBUTION OF T. wallichiana IN H.P.

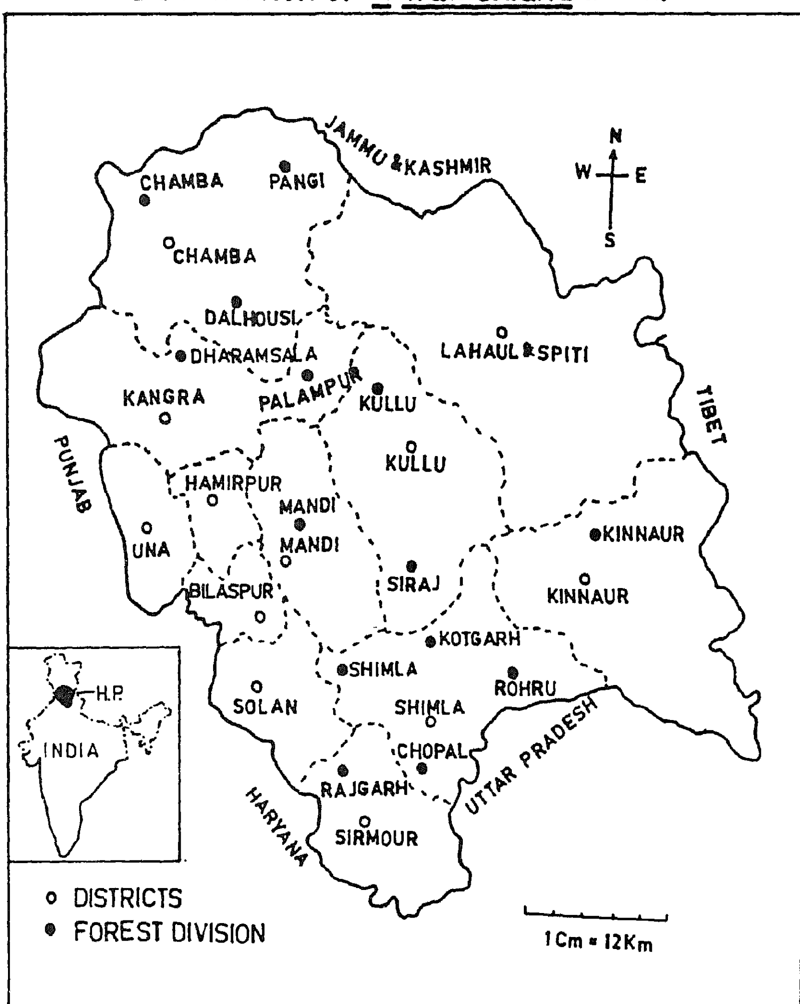


Figure 2. Distribution of *Taxus wallichiana* in different forest divisions of Himachal Pradesh

recent. The regeneration is almost absent except in a few inaccesible areas. The potential areas of *Taxus* which sustained it a few decades ago are now devoid of this plant. Little is known about its genetic diversity. It has been a plant of insignificance to forest departments so far. For its long term health and sustainable utilization, work on; selection and cultivation of elite desirable clones and conservation of natural stands assumes immediate priority. The availability of taxanes on sustainable basis can be assured by harvesting clippings from close density plantations maintained in bush form. The clippings of Himalayan yew contain small amounts of taxol, but higher levels of its precursor baccatin III besides other taxanes (Miller *et al.*, 1981; George *et al.*, 1993). As taxol is no more a drug of choice and many other semisynthetic taxanes like taxotere (Colin *et al.*, 1988; Bissery *et al.*, 1991; Gueritte *et al.*, 1991) show better patient acceptance, the availability of baccatin III becomes all the more pertinent than taxol, as it can be converted to such acceptable taxanes with lesser side effects, by esterification reaction (Denis *et al.*, 1988; Mangatal *et al.*, 1989) or other alternatives (Palomo *et al.*, 1990; Swindell 1991). The overall recovery of baccatin III can further be enhanced by chemical process of other taxanes in the crude extracts of the clippings.

Regeneration and Vegetative Propagation

Keeping the aforesaid in view work was initiated by Regional Research Laboratory, Jammu at its Extension Centre, Palampur (HP) on mass macro-propagation. Based on survey and screening, the elite plants from natural stands are being clonally propagated with the aim to grow *T. wallichiana* plants in field conditions in close density bush type plantations. The clippings obtained from such plantations can be harvested on sustainable basis without affecting the natural reserves in the forests. The following techniques are being employed for mass propagation after proper optimization:

(i) Propagation through Cuttings

The maximal rooting in *T. wallichiana* depends on (a) type of cuttings (b) health of the mother plant (c) season and (d) rooting conditions e.g., rooting medium, light, temperature, etc. Though induction of rooting was achieved in all types of cuttings (age groups), the maximum rooting was obtained in terminal cuttings (main axis) 80-90%, followed by 2nd year growth 65% and 3rd year growth about 40-50% after IBA treatments. The main axis cuttings are preferred for induction of rooting due to their excellent subsequent shoot extension growth. The cuttings are treated with 100-200 ppm IBA for 18 hrs or a quick dip in 1000-2000 ppm solution and planted in previously prepared plastic tubes or field beds under partial shade. As reported in other species of *Taxus* (Kim and Nam 1985, Eccher 1988) IBA only helps in enhancing rooting process in *T. wallichiana* also. The root growth is best obtained by making the rooting medium light by mixing thermocol granules and sand. The best season for induction of rooting was found to be November to February. The plants are ready for transplantation with 120 to 180 days depending upon the time

of planting. Rooting response was drastically reduced after April under long day conditions.

Some workers (Studebaker *et al.*, 1988, Ball, 1987) have suggested bottom heat for better results. However, the high cost technique was circumvented by planting the cuttings during winter at lower altitudes. Effective soil sterilization, drainage and use of Bavistin soil drenching is essential to avoid root rot and other infections.

(ii) Air-layering

Through this technique plants can be prepared in about 90 days for transplantation. The air layering is conducted on selected elite plants using green moss and IBA. The number of plants produced through this technique is limited, nevertheless, the plants produced are comparable to 3-5 years old plants raised from seeds. This technique can be gainfully used for producing mother stocks from elite trees with high taxane content.

(iii) Seeds

The propagation through seeds is time consuming due to their double dormancy (Wyman 1971) and slow growth of plants to reach clipping stage. The seeds of *Taxus* required stratification and GA_3 treatments to achieve maximal and earlier germination. Usually it takes one year for the seeds to germinate (Suska, 1985). Propagation through seeds is being mainly adopted in our laboratory for introduction of exotic cultivars and species for their evaluation under local conditions. So far we have introduced *T. baccata* (European yew), *T. brevifolia, T. cuspidata*, and *T. chinensis*.

Cultivation

Taxus can grow in very poor soils once established and does not require nutrient inputs (Witt 1987). It is however highly susceptible to water logging (Chittenden 1951) and is best planted in slopy areas. We have adopted a 40 cm x 40 cm spacing for close density cultivation. The plants are susceptible to transfer shock and the transplantation is carried with minimum damage to roots. It grows well in shady areas and can establish in all types of soils from limy to acid. A model of cultivation based on mixed plantation with other tree species like Mulberry has been initiated for creating a two tier canopy model for commercial cultivation in lower Himalayas. Preplant chilling and post-plant enhanced light duration has been reported (Lumis and Johnson, 1983) to have positive effect on the subsequent growth of *Taxus* x *media* cv. Hicksii.

Chemical Considerations

The work on taxanes in *Taxus* spp. received active attention only after the discovery of anticancer activity in taxol. The chemistry of taxanes is out of purview of this article, however articles by Blechert and Guenard 1990, Suffness and Cordel-1985,

Kingston, 1991, Guenard *et al.*, 1993 give excellent details. Though some synthetic approaches have been developed (Holton, 1990; Horwitz, 1994; Nicolaou *et al.*, 1994) and till such approaches are perfected to economic viability, it is essential to understand the environmental and genetic factors which influence the composition and concentration of taxanes for proper cultivation and management of yew, towards a sustained supply of raw material.

(i) Environmental Effects

Most of the variations in taxane contents within different populations have been attributed to environmental factors. The major factor is that of light (Vance and Kelsey, 1992; Kelsey and Vance, 1992). Trees growing in shade have higher taxol content in bark, whereas the reverse is true of cephalomannine, when compared with trees growing in open. Similarly, baccatin III also behaves differently. (Wheeler *et al.*, 1992, Kelsey and Vance, 1992). Our studies (Puri *et al.*, unpublished data) on *T. wallichiana* from Himachal Pradesh reveal higher concentrations of cephalomannine and baccatin-III as compared to taxol. The plants growing in cool and moist sites have overall higher taxane contents in contrast to plants growing on warmer and dry slopes. The total taxane contents also vary with altitude and season. The optimum time of collection was found to be June-July for both bark and needles. Large variations in taxane profile have also been reported in *T. brevifolia* (Wheeler *et al.*, 1992).

(ii) Genetic Factors

Though almost all the species grown around the globe contain taxol and other taxanes or derivatives in their leaf and bark (Fett-Neto *et al.*, 1992, Kelsey and Vance, 1992; Vidensek *et al.*, 1990), they show typical differences in their taxane profiles (Witherup *et al.*, 1990) pointing to the inherent genetic differences. Significant differences in taxol concentrations among plants of same species growing under identical conditions (Kelsey and Vance, 1992), notwithstanding the differences due to season and other factors, also point to genetic variations. However, the amount of genetic variations and heretability of relevant traits are yet to be defined in *Taxus* species. A genetic test based on screening of plants raised through seeds obtained from thirty different populations of pacific yew and grown under identical conditions has been reported by Wheeler *et al.*, (1992). Significant differences were found in height, growing points, time of bud burst and bud set, between populations and among trees within populations.

Working on the allozyme variation based on 24 enzymatic loci in *T. brevifolia* and other plants, Hamrick and Godt (1990) reported lower degree of variation as compared to other gymnosperms, but comparable to other angiosperms.

The genetic selection is, however, complicated by changes in taxane contents in different seasons. In view of the lack of a reliable heirarchy in taxane levels, the selection based on maximal biomass accumulation and phenological traits, besides the overall taxane contents seems more attractive proposition. We have already

initiated a study in which different *Taxus* species obtained from different parts of the globe are being introduced to evaluate their performance under local conditions. Since taxane biosynthesis involves a dozen or more enzymes or genes (Zamir *et al.*, 1992), population and phenotypic correlation studies in carefully controlled environments would enhance the selection pressure. Such a study on Himalayan yew, with diverse ecological situations through the whole length of Himalayas would be fruitful. In view of the importance of baccatin III as precursor, selection pressure for higher baccatin-III yield needs priority.

Post Harvest Technology

In view of the limited resource availability, optimization of post harvest operations from harvest to extraction of clippings is receiving attention of late now. The taxol yields are most sensitive to drying protocol (Hansen *et al.*, 1993). A thin layer drying system (Keener 1991; Hansen *et al.*, 1993) has been suggested. The loss of taxane yields on long term storage of clippings is not worked out yet. Recently, Elsohly *et al.*, (1994) have elucidated taxol content variations in fresh, stored and dried *Taxus* clippings. It is interesting to note the findings of Schulzter *et al.*, (1994) who reported enhancement in the yield of taxol and cephalomannine on storage for nine days, regardless of storage temperature. Similarly the extraction procedures also affect the overall taxane yields. A comparison of the values of taxol and other related compounds by different workers (Witherup *et al.*, 1990; Vidensek *et al.*, 1990 and Kelsey and Vance, 1992) show 2-10 fold differences in the overall values. Notwithstanding the differences due to environmental and genetic factors, these differences have a third component attributed to post harvest procedures, storage and extraction protocol, tissue particle size, type of solvent, temperature and length of extraction which may effect the final yield.

Cell Culture and Biotechnology

Taxol production through cell cultures has been suggested as an alternate source of taxanes. Though many patents have also appeared in this respect, the yields fail to compete with yields and economy of clippings as a source. Christen *et al.*, (US Patent No.501.9504) obtained callus on Gamborg's B_5 medium supplemented with casamino acids (2g/L) obtained a yield of 1-3 mg/L after 2-4 weeks. The growth of *T. cuspidata* and *T. canadensis* callus and cell suspension cultures have been optimized on Gamborg's B_5 salts supplemeted with 1.5% (W/V) soluble polyvinylpyrrolidone 0.3% (W/V) sucrose and 4 mg/L 2,4-D (Shuler *et al.*, 1992). Work on growth, optimization, chracterization and taxol production kinetics have been done by Wickremesinhe and Arteca (1993,1994), Fett-Neto *et al.*, (1993, 1994). Most of such reports are based on the basis of addition of some additives (amino acids) and or elicitors (Stierle *et al.*, 1993). For example certain amino acids like phenylalanine can increase taxol yields as it serves as a precursor for N-benzyl

phenylisoserine side chain of taxol. An increase of taxol from 2 $\mu g/g^{-1}$ to $10 \mu g/g^{-1}$ of extracted dry weight at stationary phase can be achieved in presence of phenyl alanine and benzoic acid (Fett-Neto *et al.*, 1994). The stimulation of taxol production in callous cultures by endophytic fungi (Stierle *et al.*, 1993) and even growth retardants (Strobel, 1994) has prompted some to suggest a phytoalexin role for taxanes (Heinstein and Chang 1994).

Diseases

Taxus serves as a host to a diversity of micro- organisms some of which are highly pathogenic and pose threat to its cultivation. The Himalayan yew is yet to be investigated in this respect. In *T. brevifolia* a highly destructive root rot is caused by *Phytopthora lateralis* (Mangatal *et al.*, 1985). A lack of self-sown regeneration is caused in *T. baccata* populations infested with *Phellinus hartiqii* and *P. pini*. These pathogens along with *Cylindrocarpon radicalis* and *Mycelium radicisatrovirens* cause decay of wood (Manka *et al.*, 1986). The yew leaf blight due to *Macrophoma taxi* which cause small black perithecia under the surface of leaves is prevalent in Himalayan yew also. Removal of branches and spray of Bordeaux mixture help in controlling it. Another disease, yew leaf scorch due to *Sphaerulina taxi* causes browning of leaves.

Conclusion

The blatant exploitation of Himalayan yew in recent years has created serious concern about its long term health in the already diminishing natural populations. There is a need for developing effective strategies in management of Himalayan yew in light of its recently found utility. The harvest of leaf clippings obtained from close density bush type plantations of genetically elite plants (clonal populations) offers a sustainable and non-environmental impacting system, till some other source through biotechnology or chemical synthesis is perfected to comparative economic viability. The effective conservation and utilization of Himalayan yew requires a comprehensive study on its regeneration potential, reproductive biology, environmental impacts and genetic diversity.

Summary

The discovery of anticancer agent taxol and its related compounds in different species of *Yew* (*Taxus* spp.) has evoked worldwide interest in development of strategies for a sustainable supply of taxol. As taxol is present in miniscule quantities, its extraction from needles and bark has already resulted in a blatant exploitation of the *Taxus* from its receding natural habitats, Himalayan yew being no exception. The biology, cultivation, conservation, utilization of *Taxus* including its post harvest aspects are discussed here in view of its newly found importance and world demand. The need for exploiting genetic variation to build a strong

selection base for enhanced biomass and taxane yields, coupled with recent developments in its agrotechnology are emphasised. It is suggested that besides selection of *Taxus* genotypes from natural habitats in India, germplasm from different sources be collected and potential high yields be vegetatively propagated through cuttings, seed and tissue culture on a mass scale both for reforestation and bush type plantation, notwithstanding biotechnological approaches being pursued at the moment for taxol production.

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Chemistry and Biological Activity of Aescin

Bikram Singh*'& P.K.Agrawal+

C.S.I.R Complex, Palampur - 176 061
Himachal Pradesh (India)

Introduction

THE genus *Aesculus* comprises twenty five species which grow in northern temperate region as trees and shrubs[1]. Only six species namely, *A. hippocastanum, A. turbinata, A. indica, A. punduana, A. glabra* and *A. pavia*, have been chemically and biologically investigated.

The seeds of these plants, known as horse chestnuts, are used as food, feed and fodder[2-16] and for the production of alcohol[17-24]. They possess therapeutic importance in human and veterinary medicines[25,26], e.g. in the treatment of fevers, for the uses of piles, obstinate constipation[1], of mammary induration and cancer[27]. The meal of horse chestnuts is employed to cleanse oily skin[28]. Due to their saponin content, the seeds possess toxic properties[29].

Alcoholic extract of seeds of *A. hippocastanum* shows hemolytic, antiedema and other pharmacological properties[30-55]. Various applications of this extract are reported in Table 1. It has been also applied in cosmetics[34] whereas the oil extracted from *A. indica* seeds exhibits a significant anti-inflammatory activity in carrageenin induced edema in rats[35].

Sometimes, addition of other plant extracts or chemical compounds enhances their activity or causes a change in biological effects (Table 2)[56-68].

*Author to whom correspondence may be made

+Central Institute of Medicinal & Aromatic Plants, Lucknow-226015, India

Table 1—Biological activity of the horse chestnut EtOH extract

S. No.		Activity	References
1.		Capillary permeability activity	
	(a)	In relation with physiology, pathology of the venous presure	36, 37, 38
	(b)	Provide resistance to skin capillaries	39
	(c)	Effect on the pathology of blood coagulation	40
	(d)	Effect on cerebral circulation and metabolism	41
	(e)	Effect on peripharal vascular system	42
	(f)	Changes in arterial saturation with oxygen	43
	(g)	Increased capillary fragidity in hypertension and hemorrhagic diathesis	44
2.		For various skin disorder and diseases via cutaneous application	45
3.		For the treatment of rheumatism, arthritis and neuralgia	46
4.		Decreases the permeability of blood vessels to protein and brought the albumin/globulin ratio closer to normal	47
5.		Action with respect to the effect on stomach intestine, urinary bladder, uterus, heart, blood pressure and respiration	48
6.		Increases the tonus of isolated intestine	49
7.		Inhibition of Ehrlich ascites carcinoma in mice	50, 51
8.		Inhibits edema induced by histamine in rats and guinea pig	52
9.		Antiarthritic activity in pigs	53
10.		Inhibition of hyaluronidase	54
11.		Employed in whooping cough	1
12.		Diuretic effect on cardio-vascular system	55
13.		Spasmolytic activity	55

Table 2— Biological activity of the horse chestnut EtOH extract mixed with other substances

Other substances	Activity	References
Mountain ash (*Sorbus aria*)	Raise the capillary resistance and have antiphlogistic effect	56
Vitamin B	Cause tightening of the membrance and effect on the circulation	57
Glutamic acid	For the treatment of phlebitis	58
Hamamelis virginiano and Rutin	Used as a venotropic ointment, active against varicose vein and hemmorrhoids	59
Witch-hazel, zinc stearate, sweet almond oil and calendula	Useful for cleansing hemorrhoidal area of human body	60
Hamamelis, sage essential oil and menthol	Cream particularly for legs	61
Witch hazel, sulphur, fragon extract and clove oil	Softening action on the skin to relieve the tiring sensation in feet and legs caused by poor blood circulation	62
Hyaluronidase	Cosmetic preparation for treatment of callulitis and S.C.accumulation of fat globules	63
Paraphenyl-bis (β-hydroxyethyl) benzylaminochloride, Vitamin-A, inositol, Vitamin-B complex and *Iso*-propyl adipate	Hair treatment composition	64
Stearic acid, lecithin, triethanolamine, glycerol and vaselin	In cosmetics, inhibit the chilblain and wrinkles, prevalent atrophy & elasticity reduction of skin and inhibit inflammation caused by dextran or carrageenin	65
Fatty alcohol, sulfate, Poly (vinyl alcohol), acetyl alcohol, and glyceryl laurate	As cosmetic and shampoo for hair	66
Viscosity-increasing water glass (d 1.32) and sodium polyphosphate	Fire extinguishing foams	67
Rutin and azulene	Effect on skin permeability	68

Chemistry of Aescin

The presence of saponin in *A. hippocastanum* was reported for the first time by Fremy about one and half century back[69]. This saponin was, later on, isolated by several research workers[70-78] and Van der Haar named it 'aescin'[79]. It was obtained from *A. hippocastanum* in a crystalline form by several research groups[80-82] and later on from other *Aesculus* species[83-87]. Finally, it was reported to be a complex mixture of several saponins[88,89].

Ruzicka *et al.*, suggested that the aglycone moiety of the saponin belongs to the pentacyclic triterpene group[90]. Kuhn and Row carried out hydrolytic studies and separated five aglycones [91], out of which one component, named *aescigenin*, was assigned the structure of 22β, 24, 28-trihydroxy-16α, 21α-epoxy-β-amyrin[92]. The hydroxyl group orientation at C-22 was, later on, established as , α as shown in structure (*1*), which was moreover confirmed by X-ray crystallography (Fig. 1)[93,108].

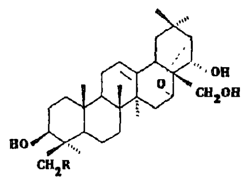
Romisch *et al.*, on the basis of hydrolytic studies, concluded that two moles of glucose and one mole of glucuronic acid constitute the glycosidic portion[80]. In addition to the above sugars, galactose and xylose may co-occur as sugar components of glycosidic moiety of aescin[94], however glucuronic acid is the internal sugar being substituted with other sugars and bonded to the aglycone in C-3 position[88-95].

Gas chromatography analysis of the products obtained by alkaline hydrolysis of aescin showed also the presence of acyl groups deriving from *acetic* (1 mole), *isobutyric* (0.1 mole) *α-methyl butyric* (0.15 mole), *angelic* (0.35) and *tiglic* (0.4 mole) acids[80,88,89,96].

Based on hydrophylicity and hemolytic activity, Wagner *et al.*, suggested three forms of aescin, namely *α-aescin (2)*, *β-aescin (3)* and *crypotoaescin-A(4)*[96-98]. The physical constants for all the three forms are given in Table 3.

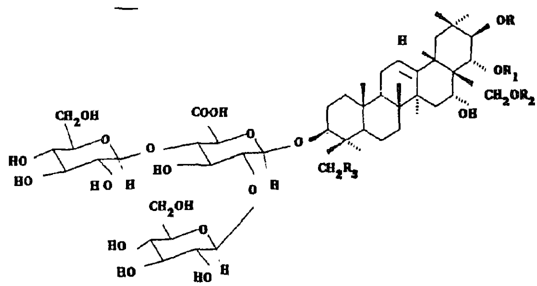
Tschesche *et al.*, in 1963, elucidated the structure of the major saponin of β-aescin as 3-0-[β-D-glucopyranosyl(1?2), β-D-glucopyranosyl-(1?4)]- β-D-glucuronopyranosyl- 3β, 16α, 21β, 22β-pentahydroxy, 21-angeloyl, 22-acetyl-olean-12-ene(*5*) on the basis of chemical degradation and spectral analysis[88]. These authors also suggested that aescin is a complex mixture of several saponins differing in sugar constituents, aglycone and in having the site of esterification which may be C-21 or C-22 (Table 4). The site of glycosidation in all cases was revealed at C-3 position[95].

Later on, Kuhn and Loew isolated *protoaescigenin* from the acid hydrolysate of aescin and elucidated its structure as 16α, 21α, 22β, 24, 28-pentahydroxy-β-amyrin. The stereochemistry of the hydroxyl group at C-21 and C-22 as proposed by Kuhn and Loew was reversed and the revised structure of *proteae-scigenin* was considered as 16α, 21β, 22α, 24,28-pentahydroxy-β-amyrin (*6*)[89,90]. The structures of two further sapogenins also present in aescin *aescinidine* (Barringtogenol C) and



1 Aescigenin R = OH

12 Barringtogenol-D R = H



2 -Aescin : R = acyl group, R_1 or R_2 = $COCH_3$, R_2 or R_1 = H, R_3 = OH

3 -Aescin : R = acyl group, R_1 = $COCH_3$, R_2 = H, R_3 = OH

4 Cryptoaescin-A : R = acyl group, R_1 = H, R_2 = $COCH_3$, R_3 = OH

5 Major component of -aescin : R = angeloyl, R_1 = $COCH_3$, R_2 = H, R_3 = OH

9 Aesculuside - A : R = Angeloyl, R_1 = R_2 = H, R_3 = OH

10 Aesculuside - B : R = R_1 = R_2 = H, R_3 = OH

11 R = R_1 = angeloyl, R_2 = R_3 = H

Acyl groups

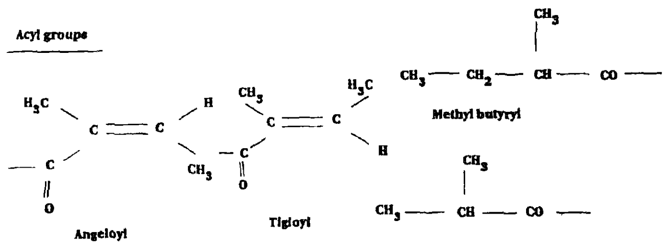


Fig. 1 Structures of sapogenins and saponins of aescin

Table 3—Physical constants of different forms of aescin

Aescin form	mp	$[\alpha]_D$	Solubility in H_2O	Hemolytic activity
α-Aescin	220-2°	-13.5°	Soluble	1:10, 000
β-Aescin	224-5°	- 28.5°	Sparingly soluble	1:40,000
Cryptoaescin-A	218-20°	- 4.0°	Soluble	Inactive

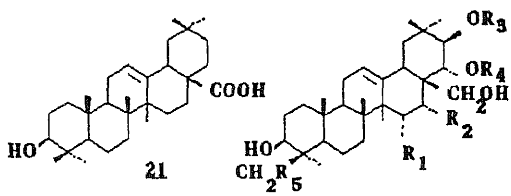
Table 4—The components of aescin (*A. hippocastanum*)

Triterpene	Sugar	Acid	% in aescin
Protoaescigenin	D-glu.A, D-glc, D-glc	Angelic acid acetic acid	23
	D-glu. A, D-glc, D.glc	Tiglic acid acetic acid	15
Protoaescigenin	D-glu.A, D-glc, D-xyl	Angelic acid acetic acid	9
	D-glu.A, D-glc, D-xyl	Tiglic acid acetic acid	6
	D-glu.A, D-glc, D-gal	Angelic acid acetic acid	7
	D-glu.A, D-glc, D-gal	Tiglic acid acetic acid	5
Barringtogenol-C	D-glu.A, D-glc, D-glc	Angelic acid acetic acid	5
	D-glu.A, D-glc, D-glc	Tiglic acid acetic acid	3

*D-glu.A = β-D-glucuronopyranoic acid;
D-glc = β-D-glucopyranose;
D-xyl = β-D-xylopyranose;
D-gal = β-D-galactopyranose.

isoaescige-nin earlier identified as 16α,21α,22β,28-tetrahydroxy-β-amryrin[100-103] and 21α, 22β, 24,28-tetrahydroxy-β-amyrin[104] were later on found to possess β- and α-orientations of the C-21 and C-22 hydroxyl group respectively as shown in structure (7) and (8)[95,102-107].

The acid and enzymatic hydrolytic studies on aescin obtained from various *Aesculus* species yielded different aglycones (Table 5), concluding that aescin is a complex mixture of several glycosides possessing different aglycones (*1*, 6-8, 12-24). These investigations have been performed in aescin isolated from *A. hippocastanum*[109,111], *A. turbinata*[109,112], *A. glabra*[113], *A. punduana*[114] and *A. pavia*[115].



Sapogenin	R1	R2	R3	R4	R5
6. Protoaescigenin	H	OH	H	H	OH
7. Aescinidine (Barringtogenol C)	H	OH	H	H	H
8. Isoaescigenin			H	H	OH
13. 16-desoxy Barringtogenol C	H	H	H	H	H
14. 21-angeloyl, 22-tigloyl or 21-tigloyl, 22-Angeloyl-R1-Barringenol	OH	OH	Angeloyl or Tigloyl	Tigloyl or Angeloyl	H
15. 21-Angeloyl Barringtogenol C	H	OH	Angeloyl	H	H
16. R1-Barringenol	OH	OH	H	H	H
17. 21,22-diangeloyl-Barringtogenol C	H	OH	Angeloyl	Angeloyl	H
18. 21,22-diangeloyl-R1-Barringenol	OH	OH	Angeloyl	Angeloyl	H
19. 21-angeloyl-R1-Barringenol	OH	OH	Angeloyl	H	H
20. 21-tigloyl-Protoaescigenin	H	OH	Tigloyl	H	OH
22. 21-angeloyl-Protoascegenin	H	OH	Angeloyl	H	OH
23. 21,22-diangeloyl-Protoascegenin	H	OH	Angeloyl	Angeloyl	OH
24. 21-tigloyl-Barringtogenol C	H	OH	Tigloyl	H	H

Fig.2 Structures of sapogenins of aescin

Table 5: Sapogenins formed by acid and enzymatic hydrolysis of aescin

A hippocastnum acid[109,111]	Enzymatic acid	*A Turbinate*[109,112]	Enzymatic	*A.Glabra* acid[113]	Enzymatic	*A.punduana*[4] Acid	Enzymatic	*A.payia* acid[115]	*A.indica* acid[110]
(*6*)	(*6*)	(*6*)	(*6*)	(*7*)	(*7*)	(*7*)	(*7*)	(*19*)	(*6*)
(*1*)	(*7*)	(*1*)	(*7*)	(*16*)	(*18*)	(*20*)	(*20*)	(*15*)	(*1*)
(*7*)	(*8*)	(*7*)	(*13*)		(*15*)	(*21*)	(*21*)	(*22*)	(*7*)
(*12*)	(*13*)	(*12*)			(*19*)			(*23*)	(*15*)
(*13*)		(*13*)						(*24*)	(*17*)
(*14*)									(*19*)
(*15*)									

Table 6— Biological activity of aescin

S.No		Activity	References
		Antiedema effect	
1.		Effect on rat paw edema induced by :	
	(a)	dextran	120-128
	(b)	egg white	126, 129
	(c)	formalin	126, 127, 130
	(d)	Ovalbumin	125, 127, 131
	(e)	carageenin	123, 125
	(f)	bradykinin	125
	(g)	buphenin	132
2.		Mechanism of action against rat paw edema	124, 125, 127
3.		Effect on rat brain edema induced by triethyltinsulfate	133-142
4.		Mechanism of action against brain edema	
	(a)	in relation with change of water contents of cerebral substances	135
	(b)	in relation with increased cerebrospinal fluid pressure in the cistera magna	133, 134
5.		Antiedema effect on rabbit central nervous system	143
6.		Antiedema, study on the dialyzability	144
		Anti-inflammatory activity	
7.		Against the inflammation caused by	
	(a)	kaolin	125
	(b)	formalin	125
	(c)	bee poison	125
	(d)	foreign bodies	147, 148
8.		Effect on granuloma in mice elicited by inplantation of cotton pellets and formalin	122
9.		Mechanism of anti-inflammatory activity in relation with adrenohypophyseal axis	126
10.		Comparative study on anti-inflammatory action caused by aescin and phenyl butazone	149
11.		Effect of the cytology of inflammatory exudate *in vitro* & *in vivo*	150
		Effect on circulatory system:	
12.		Effect on human saphenous vein	151, 152
13.		Mode of action and release of prostaglandin-F_2	152, 153
14.		Biochemical function in the illness of the venous system	154
15.		Preventing effect and mechanism of action on the vasodilation caused by histamin-HCl	155

(Contd.)

S.No		Activity	References
16.		Capillary permeability action with respect to lymph flow	156
S.No.		Activity	References
17.		Pharmacodynamic result with respect to capillary permeability	157
18.		Enhancement of capillary permeability action of the skin	158
19		Purcutaneous absorption in skin of rat	159
20.		Importance in cosmetics due to improvement in circulation	160
21.		*Haemolysis*	
	(a)	of erythrocytes	162, 164
	(b)	haemolytic action in relation to:	
	(i)	the increase in concentration of lectic dehydrogenase isoenzyme	165
	(ii)	of blood buffer soln. at pH 10	161
22.		Greater haemolytic activity and much less anti-coagulant activity	166, 167
23.		*Antifungal activity*	
	(a)	against mycelium *Botrytis cinera* and *R. solana*	170
	(b)	cytostatic activity against *Tricho-derma menlagrophytes*	169
	(c)	Fungistatic activity on dermatophytes	170
	(d)	Inhibitor of fungal growth metabolism	171-173
24		Various reviews regarding, toxicity detoxification, haemolytic activity, local tolerance, blood pressure respiration, cosmetic, therapeutic and pharmaceutical values	174-177
25.		Anti-cancerous activity on Ehrlich mouse ascites carcinoma	178-181
26.		Corticomimetic activity against cold in adrenalectomized rats	182, 183
27		Hypotensive action, toxicity to rats and range hypotension, accelerate respiration, acts as cardiac stimulant	184, 185
28.		Activity on prostrate and seminal vasicle in rats	186
29.		Antiherpes activity	187
30.		Inhibiting and reducing effect on the development of atherosclerosis	188
31		Inhibiting effect against radiation in male & female rats	189
32.		Damaging effect on termites	190
33.		Toxicity and activity in rats	191-193

Table 7— Activity of the derivatives of aescin

S. No.	Derivatives of aescin	Activity	References
1.	Sodium aescinate	Anti-inflammation caused by foreign body	194
2.	1-Benzyl-3-(3-dimethyl-amino-propoxyl)-1-indazole aescinate	Anti-inflammatory anti-exudative and analgesic agent	
3.	Sodium-aescinate	Haemolytic	196
4.	Aescin-methyl ester and aescin-ethyl ether	Haemolytic	197
5.	Sodium aescinate	Effect on brain edema in rabbit	198
6.	Sodium aescinate	No effect on human kidney function	199
7.	Sodium aescinate	Its activity in amorphous form	209

Table 8— Biological activity of aescin mixed with other substances

S. No	Other substances	Activity	References
1.	Calcium hydroquinone sunfonate	Used to treat edema and varicose vein	201
2.	Hesperidin, methyl chalcone, trihydroxyethyl rutoside and billerry anthocyanosides	Anti-edema effect against ovalbumin and anti-inflammatory action against the inflammation caused by foreign body	202
3.	Tri-(hydro-ethyl)-rutoside and Vitamin C	Protective anti- edema effect in patients with posthrombic edema	203
4.	1-Phenyl-2, 3-dihydro-4-(bicyclo[2, 2, 1]-hept-2-ylamino)-3-pyranoline-5-ene	Used as antiphlogistic, antineurolgic and anti-exudative activity against edema in rat paw carrageenin	204
5.	Dexamethasone	Edema inhibiting effect against ovalhumin induced edema	205
6.	Buphenin	Inhibition of edema by cutaneous application to rat paw kaolin edema	206

(Table 8 Contd.)

7.	*P/O*-hydroxybenzoic acid ester rutin panthenol, nicotinamide Vitamin-E	Treatment of periodental inflammation (mouth care)	207
8.	Flavonoids	Significant anti-inflammatory action against an anaphylactic edema in rats	208
9.	3, 5, 3′-tri-iodothy-rocarboxylic acid	Active against cellutitis and vein edemas	209
10	Acetazolamide, mucopolysaccharidase and optionally hyaluronidase	For treating fat infiltration and cellulitis	210
11.	Mucopolysaccharidase optionally hyaluronidase with 3, 5, 3′-tri iodothyroalkanoic acid as their salt or ester aceta-zolanide	Used for the treatment of obesity and cellulitis	211
12.	Troxerutin and bupenin hydrochloride	Anti-inflammatory and low toxicity	212
13.	Heparin-sodium ethylene glycol monosalicylate	Inhibition of S.C. exudate formation after S.C. inplantation in rats by tropical application	213
14.	Papaverine and dihydroergocristine	Stimulated cerebral and peripheral circulation increasing venous blood pressure	214
15	Indomethacin	Antagonization effect on plasmalymph barrier of rabbit caused by prostaglandin E_1 (PGE_1)	215
16.	Cyclonamine	Effect on capillary permeability in rats	216
17.	Cyclonamine	Effect on capillary resistance and coagulation is guinea pigs	217
18.	Diosmin, calcium dobesilate, ethamsylate and folescutol	Effect on increased capillary permeability	218
19.	Cortisone	Effect on lumphatic permeability in the rats	219
20.	Dihydroergostamine methane sulfonate, rutoside and ascorbic acid	For systematic treatment of venous disorder	220

(Table 8 Contd.)

21.	Avenacin	As inhibitor of fungal growth and metabolism	221-225
22.	α-globulin, lactose, talc starch, sucrose, poly (vinyl pyrolidinone and magnesium)	Effective for curing acute haemorrhoids	226
23.	O-(β-hydroxyethyl) rutoside	Inhibited the hemolytic action of various amphilic substances on human red blood hemolysis	227
24.	β-Acetyldigoxin	Biological activity of oral pharmaceutical formulation and *in vitro* test	228
25.	Gitoxin	Showed improved bioability	229
26.	Aesculin, glycyrrhizic acid, β-sitosterol and sage oil	As an antiperspirant	230
27.	Sterol complex	Pharmaceuticals for local application	231
28.	*P*-diphenylalanin	For the improvement in rabbits spinal cord injury	232
29.	Mucopolysaccharide	Accelerates and prolongs the body weight reducing action of mucopolysaccharide	233
30.	Resorcinol, β-hydroxyquinoline, pyridoxin ethavarine prednisolone and tetracycline	As indicator in semen detction	234
31.	Rutin and ergot alkaloids or their methane sulfonate	For the treatment of infection diseases of ear, nose and throat, vocal cord inflammation, heavy hoarseness and thrombophlebitis	235

During our chemical investigation on *A. indica* we found that the total content of 'aescin' is about 8% with respect to dry weight of the seed and 13% in *A. hippocastanum*[116].

The aescin of *A. indica* is a mixture of four components, out of which two are predominant. Column chromatography lead to the isolation of the major constituents, designated as aesculuside- A(9)[117] and aesculuside-B(10)[118]. Recently, a new

molluscicidal. triterpene glycoside (*11*) has been isolated from *A. indica*[119]. The minor saponins could not be isolated.

The biological and pharmacological significance of aescin is compiled in Table 6. Aescin possesses remarkable antiedema and anti-inflammatory effects and enhances capillary permeability. Due to the latter property, it is widely employed in cosmetics[120-193].

Derivatives of aescin have been also reported to possess therapeutical importance[194-200] among which the sodium salt, commercially known as 'Reparil', has been mainly used. Table 8 sumarises the activities exhibited by aescin derivatives. It has been also noted that addition of other chemical component(s) to aescin sometimes enhance its therapeutical value[201-235].

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Plantago ovata Forsk.: Cultivation, Botany, Utilization and Improvement

A.K. Koul & S. Sareen

Department of Biosciences
University of Jammu, Jammu, Tawi - 180 001

PLANTAGO OVATA Forsk., the source of Isabgol or blond psyllium, belongs to genus *Plantago*, which is one of the three genera comprising family Plantaginaceae. The other two are *Littorella* Bergius and *Bougueria* Decne. Of the 281 species of *Plantago*, only two species, namely *P. ovata and P. psyllium* (French psyllium) are cultivated for their seed husk which is used in pharmaceutical and cosmetic industries. The seed husk of *P. ovata* is superior in terms of swelling qualities and colourlessness for which reasons it has displaced French psyllium from the world market.

Plantago is a Latin word meaning sole of the foot which refers to the shape of psyllium leaf. Psyllium in Greek means flea; the name refers to the colour, size and shape of seeds. Isabgol is combination of two Persian words "Isap" and "Ghol" meaning horse-ear. It bears reference to boat shaped seeds.

Plantago ovata is an annual herb abounding warm temperate sandy regions between 26-36°N, latitude (Fig.1) (Stebbins and Day, 1967). The species is indigenous to Mediterranean region and W. Asia extending upto West Pakistan (Singh and Virmani, 1982). The plant is distributed from Canary islands across southern Spain. North Africa, Middle East, Pakistan and contiguous areas of North-Western Asia (Duthie, 1960; Hooker 1885; Luthra, 1950; Stebbins and Day, 1967).

Initially, the seed used to be collected from wild populations. As the natural sources depleted and the demand for seed increased, the plant was brought under cultivation. In undivided India, the plant is believed to have been introduced during

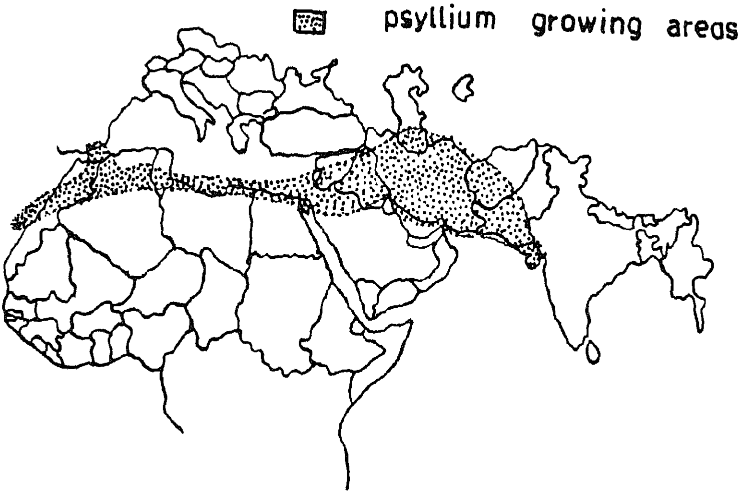

Fig 1– Psyllium growing areas of world and India

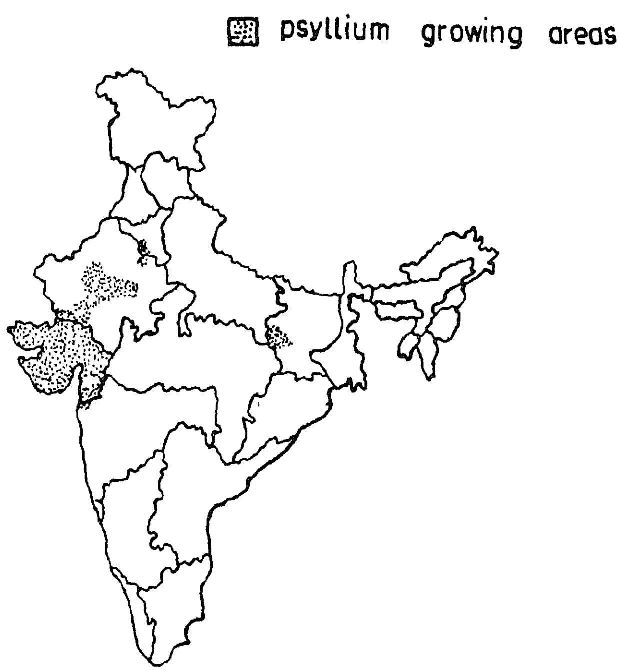

Fig. 2– Psyllium growing areas of world and India.

the Mughal rule (Husain, 1977). Its cultivation was originally confined to Lahore and Multan (now in Pakistan) and then it spread to Bengal, Mysore and Coromandel coast (Husain, 1977). At present, Gujarat is the major psyllium growing state in the country (Fig.2). In N.Gujarat, it is cultivated in Patan, Sidhpur and Kheralu talukas of Mehsana district and Palampur, Vedgaun, Deesa and Deoda of Banaskantha district (Shah, 1981). Recently, new areas in Kutch, Jamnagar, Ahmedabad, Rajkot, Junagarh, Bhavnagar, Surat, Surendranagar, Baroda, Gandhinagar, Panchmahal, Sabarkantha and Kaira have been brought under the cultivation of this crops (Mathur, Rangarajan and Gupta, 1990; Virmani, Singh and Husain, 1980). In Rajasthan the plant is grown in five districts — Jalore, Sirohi, Ajmer, Pali and Jodhpur. On account of the growing world demand, cultivation of psyllium has been extended to few other states, namely Haryana, Bihar, U.P., Panjab and Maharashtra.

According to the Agricultural Census for 1980-81, the total area under psyllium cultivation in India is 27,099 hectares. Of this, more than 27,000 hectares fall in Gujarat (Mathur, Rangarajan and Gupta, 1990).

India holds monopoly in the world trade of psyllium. More than 90 % of the total Indian produce is exported to Afghanistan, Bahrain, Bangladesh, Belgium, Canada, Dubai, Ethiopia, France, Germany, Kenya, Kuwait, Malaysia, Nepal, New Zealand, Oman, Saudi Arabia, Somalia, Sri Lanka, U.K. and U.S.A. The earnings run into crores. Exports made during 1990-91 earned the country foreign exchange worth Rs. 638.014 million (Monthly Statistics of Foreign Trade of India, Vol.I (1992).

Cultivation

Sowing Time: Psyllium is a winter season crop. Seeds are sown anytime from mid-October-December end. In Gujarat, where the crop is cultivated commercially sowing starts soon after Dassera i.e., October end, and continues till middle of December (Joshi and Tahilani, 1956). However, sowing during first week of December is considered ideal (Kalyansundram *et al.*, 1984). Early sowing, especially with higher seed rate, makes the crop vulnerable to downy mildew. Late sowing cuts short the period required for full growth, and endangers the plants with spring rains that cause seed shattering. In Pilwai tract of North Gujarat sowing during second half of November is considered ideal (Mehta, Modi and Gupta, 1976) Same is also true for Patiala in Punjab. However, in Ludhiana sowing is preferred towards end of October (Randhawa *et al.*, 1978). In Kumaon hills, trials were made to raise two crops of psyllium a year; one in November and the other in July (Joshi, Tiwari and Yadava, 1970). At Jammu, sowing between mid-October and mid-November is ideal. Late sowing, when winter rains are over, adversely tells upon seed yield due to short growth period.

Soil: The crops can be raised on variety of soils for which reason it is considered hardy. However, well-drained sandy-loam to rich loamy soil is best suited for its cultivation. The soil *p*H ideal for psyllium cultivation is 7.2-7.9 (Modi, Mehta and Gupta, 1974). Heavy soils with poor drainage (Kalyansundram *et al.*, 1984) or salinity (Pal, Jadaun and Parmer, 1988) do not suit the crop. Still, attempts have been made to cultivate isabgol in saline-alkaline soils and in hydroponics (Mandy, 1970).

In Gujarat, no sooner the Kharif crop is harvested, land is irrigated and subjected to 4-6 ploughings. It is then planted to level and press it. The field is divided into plots of convenient size depending upon texture of soil, gradient of the field and irrigation facilities available.

Seed Treatment: Psyllium seed loses viability in storage. Therefore, the seed harvested from the immediate preceding crop is preferred for sowing. The seed rate followed in the country varies from 4-13 kg/hectare. Treatment of seed prior to sowing with tetramethyl thiuran disulphide (TMTD; Thiram) or any other mercurial seed dressing @ 3 g/kg of seed is recommended. Presoaking of seeds protects seedlings against the damping off disease (Kalyansundram *et al.*, 1984).

Since the seed is small and light, it is mixed with fine sand or sieved farm yard manure before it is broadcast. This ensures dense population and high seed yield (Mehta, Modi and Gupta, 1976). No sooner sowing is over, the beds are gently swept with a broom whereby seeds get covered with soil. The broom is moved gently and in only one direction to avoid deep burial of seed.

Irrigation: Sowing is immediately followed by light irrigation. The seeds germinate within 6-7 days. In case however, they do not, second light irrigation becomes necessary. Once the seeds germinate, the beds are irrigated only when it is necessary. In all 6-8 irrigations are sufficient. Modi, Mehta and Gupta (1974) rate irrigation requirements of psyllium as medium, but Randhawa *et al.*, (1985) grade it as very low. However, frequent watering after transplantation establishes the seedlings better (Mc Neil, 1989).

Fertilizer Application: Psyllium has very low nutrient requirement. Ordinarily, 25 kg N/hectare and 25 kg P/hectare are applied as basal dose during the last ploughing. After 30 days of sowing, 25 kg N/hectare is top dressed. Kalyansundram, Patel and Dalal (1982) hold that the crop has low N requirement which is adequately met, if the field is first cultivated with a leguminous crop. If the quantity of N applied is increased from 0-50 kg/hectare, it induces reduction in N concentration and swelling factor of seeds. Randhawa *et al.*, (1985) report increase in seed yield following increase in nitrogen application. In Tarai area, highest seed yield has been recorded following application of 40-80 kg N/hectare (Singh and Nand, 1988). Higher levels of salinity, accumulation of Na and deficiency of P, Ca and Mg are deleterious to seed yield.

Weed Control: The first deweeding of psyllium fields is undertaken within 20-25 days of sowing; 2-3 weedings are required in two months. Manual deweeding

proves very costly. As a substitute three alternatives have been tried for controlling weeds. These are, (i) high seed rate (12 kg/ha), (ii) pre-plant application of 1.5 kg diuron (N'-3,4-dichlorophenyl-N,N-dimethyl urea) per hectare, and (iii) application of 0.1 kg paraquat (1,1'-dimethyl- 4,4'bipyridinium ion) per hectare, 28 days after transplantation (McNeil, 1989). Isoproturon, applied @ 0.5 kg/hectare also controls weeds of psyllium fields effectively (Patel and Mehta, 1990).

Harvesting and Yield: In *P. ovata* flowering starts two months after sowing, and the crop is ready for harvesting in March or April. Irrigation is stopped a fortnight before harvesting. Plants are cut with the help of hand sickles 15 cm above ground level. They are heaped, dried for 2 days, and then threshed in the threshing yard. As a result, the seeds separate and fall apart. They are collected and winnowed repeatedly to separate out the undesirable plant parts. There after, the seed is marketted and straw fed to cattle.

The average seed yield in psyllium is 1 tonne per hectare (Modi, Mehta and Gupta, 1974). Climatic conditions during last phase of the crop in the field affects seed yield immensely. Rain, even if mild, is highly detrimental.

Application of growth regulators has been recommended for boosting seed yield in psyllium. Spraying plants during spike emergence with 100 ppm solution of indole-3-butyric acid (IBA) steps up seed yield by 38% (Tiwari and Joshi, 1972). Application of 100 ppm solution of indole-3 yl-acetic acid is known to increase seed yield by 20%. Spike count, spike size and seed weight are increased following foliar sprays of Cycocel (CCC) and tri-ido- benzoic acid (TIBA) @ 25 mg/litre.

Plant Morphology

Plantago ovata is 10-45 cm tall stemless of short-stemmed annual herb (Duthie, 1911; Hooker, 1882) (Figs 3 & 4). Leaves are borne alternately on the stem or in rosettes addressed to soil surface. Leaf count per plant varies between 40-86. Leaf is strap shaped recurved, linear, 6.0-25.0 cm long and 0.3-1.9 cm broad leaf surface is glabrous or slightly pubescent. Scapes are axillary, glabrous or slightly pubescent, 8-62 per plant, usually exceeding the length of leaves. Spikes are cylindrical or ovoid and measure 0.6-5.6 cm. Flowers are arranged on the spike in 4 spiral rows. Bracts are glabrous, broadly ovate or rotundate and concave. Sepals, four, free, concave, glabrous and elliptic; petals 4, glabrous, reflexed and white; stamens four, exserted and epipetalous; gynoecium bicarpellary syncarpous, style is filiform and shorter than the filaments, ovary bilocular with single ovule per locule. Placentation axile. Capsule ovate or ellipsoid, dehiscing along the ring of abscission tissue that develops around the capsule (Lamba and Gupta, 1981); the pyxidium splits into an upper lid and lower base.

Seeds (Fig. 5) are cymbiform, translucent and concavo-convex. The concave side is boat-shaped and the convex side is covered with a thin, white membrane. Seeds are pinkish-grey, brown or pinkish- white with a brown streak. The outer

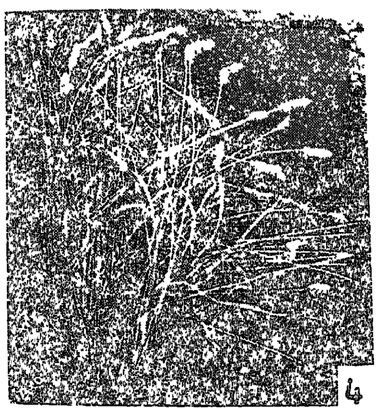

Fig 3 – Plants of *Plantago ovata* standing in field.

Fig 4 – A plant of *P. ovata.*

Fig 5 – Seeds of *P. ovata*

papery covering of the seed is termed husk; it is odourless and tasteless. When seeds are soaked in water, the mucilage contained in the husk swells (Hyde, 1970). Swelling factor of psyllium seed husk is 12.25 ml and the mucilage content is 189 mg (Sharma & Koul, 1986).

Chromosome Complement

The diploid chromosome count of the species is 8. No variation has ever been recorded in this count. The chromosome complement studied from root tip squashes comprises 2 meta- 2 submeta- and 4-subtelocentric chromosomes (Figs 6 & 7). All

subtelocentric chromosomes carry secondary constriction at distal ends of their short arms, that cut off a minute satellite. The size of secondary constriction that houses the NOR varies in the two pairs. This difference is reflected in the size of nucleoli which they organize (Sareen, Koul and Langer, 1988).

Pollen Mother Cell Meiosis: The chromosomes pair during prophase I and form 4 bivalents (Figs. 8-10). At MI most of the cells carry 2 rod an 2 ring bivalents. The Xta frequency is 1.4 per bivalent and 5.6 per cell. Chromosome segregation at anaphase is regular (Fig. 11). However, in some pollen mother cells one bivalent displays non- or delayed disjunction (Koul and Sharma, 1986). Cytomixis has been recorded in the species by Singh (1986a). It results in pmcs with variable chromosome number.

The chromosome complement as well as meiotic system have features that account for the narrow genetic base of the species; chromosome number is low (n = 4), chromosome size ranges between 2.54 and 2.92 m, chromosomes carry lot of constitutive heterochromatin, Xta frequency is low and so also is the recombination index.

Induced Tetraploidy: Tetraploidy has been induced in *P. ovata* by application of colchicine (Singh, 1986b; Zadoo and Farooqi, 1977). Details of the tetraploid

Figs 6 & 7 – Somatic chromosome complement and its photoidiogram.

Figs 8 & 9 – Stages of pmc meiosis in *P. ovata* Pmcs at diplotene (Fig 8) and diakinesis (Fig 9)

Figs 10 & 11 – Stages of pmc meiosis in *P. ovata* Pmcs at metaphase I (Fig.10) show 4 IIs and at anaphase I 4 4 segregation (Fig 11).

chromosome complement and meiosis have been published by Sharma (1984). Meiosis of the induced tetraploid is characterized by chromosome lagging, multipolar segregation and high pollen sterility.

Alterations in chromosome complement and meiosis have been induced in psyllium by application of gamma rays (Sareen, 1991). These include desynapsis, triploidy, trisomy and chromosome interchanges.

Breeding System

Genus *Plantago* includes species some of which are totally endogamous and others habitually allogamous *P. ovata* combines the two processes, which is largely due to its floral structure. Flowers of psyllium are tetramerous, actinomorphic, hermaphrodite and hypogynous. On the basis of floral structure, *P. ovata* plants are of two types. In one type, stigma protrudes out of the accessory floral whorls a day or two before anthesis (Fig. 12). In the other, stigma protrusion synchronises with the opening of flower (Fig. 13).

Stamens are epipetalous, raised 4.5 mm above the corolla tube in open flower. Anthers are versatile. The flower is bicarpellary, syncarpous. Stigma is plumose, dry and highly papillate. Mital and Bhagat (1979) hold that the stigma is entire and pointed in closed flowers but gets split into two at anthesis. This observation has not been confirmed.

Floral Biology: Blooming starts in January - February from the base of each inflorescence. The spike and scape continue to elongate even after commencement of anthesis. Anthesis is completed within 10 days for a spike and 70 days for the plant. As the flower opens, petals expand leaving gaps in between where from anthers protrude. Dehiscence of anthers takes place through lateral longitudinal slits.

Plants of one morphoform (A) are protogynous; the stigma clothed with prominent papillae, protrudes through gaps formed between corolla lobes. On the 2nd day of protrusion, stigma elongates and turns receptive. In the morphoform (B) stigma receptivity and anther dehiscence overlap. Stigma remains receptive until it is compatibly pollinated.

Pollination Mechanism: Morphoform B is autogamous. Morphoform A is pollinated by wind and insects. Plumose stigma, versatile anthers and smooth walled dry pollen grains favour anemophily. Entomophily is less common. Only *Apis dorsata* and a few dipteran flies visit the plants during the month of March when most of the spikes are in bloom. Despite anemo- and entomophily, morphoform A also is predominantly autogamous. Only the first maturing flowers on the spike indulge in cross pollination on account of weak protogyny. For rest of the flowers, pollen is supplied by flowers occupying basal region of the spike.

Male Sterile Plants: Stray male sterile plants occur in all populations. They differ from their fertile counterparts only with respect to presence of shrivelled anthers

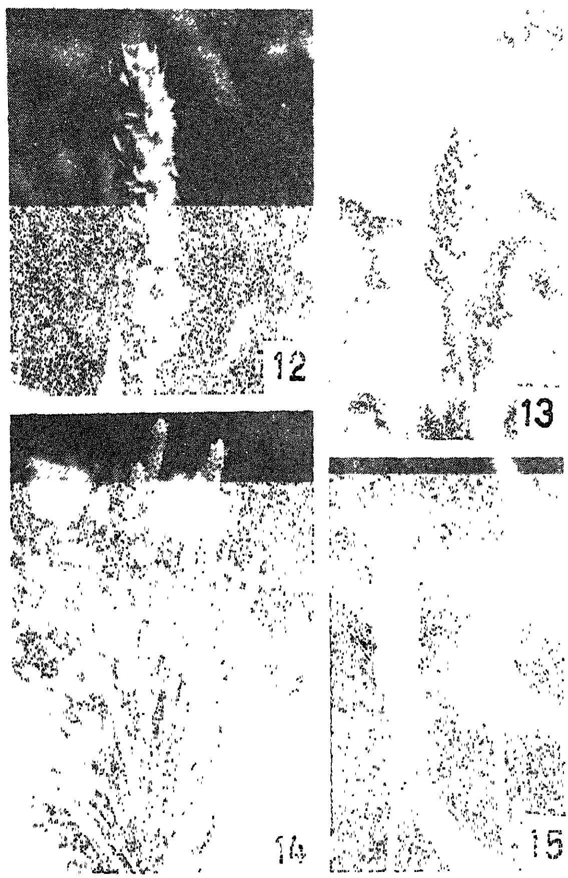

Fig. 12 – Spikes of morphoform A&B respectively

Fig 13 – Spikes of morphoform A&B respectively

Fig. 14 – A male sterile plant of *P ovata*

Fig 15 – *Plantago ovata* Forsk.: Cultivation, botany, Utilization and Improvement Male sterile and fertile spikes

(Figs 14 & 15). Male sterility is of the cytoplasmic type (Atal, 1958; Mital and Issar, 1970). Male sterile plants of the species are not visited by pollinators (Sharma, 1990) indicating that pollen is the major attraction for insects that visit psyllium flowers.

Diseases

The major fungal diseases of the crop are, wilt, damping off and downy mildews. Downymildew is the most serious disease as it causes great loss to psyllium growers. It is caused by *Peronospora plantaginis*. The disease strikes at the time of spike emergence. Chlorotic areas develop on upper leaf surface and ashy-white frost like mycelial growth on the under surface. Aureofungin has proved most effective control for the disease (Desai and Desai, 1969). Other chemicals, such as Bordeaux mixture, copper oxychloride, Dithane M-45, Dithane Z-78 etc. (Kalyansundram *et al.*, 1984) are also used to control the disease.

Wilt of psyllium is caused by *Alternaria* sp., *Fusarium oxysporum* and *F. solani* (Russell, 1975; Mehta *et al.*, 1985). Wilting starts from outer leaves and then spreads to whole plants. The leaves change their colour to silver. *Pythium ultimum* causes damping off the psyllium (Chastagner *et al.*, 1978). Fenaminosulf and CGA-48988 treatments have been recommended for protecting *P. ovata* against *P. ultimum*.

Powdery mildew attacks psyllium occasionally. The disease is controlled by use of sulphur compounds (Karthan and Sulphex). Grubs and termites damage roots of *P. ovata*. These are checked by treating the soil with Aldrin, 65% Lindane or 10% BHC. Dimethoate treatment is employed to protect psyllium against aphids.

Processing

The seed collected after thrashing the harvested plants is transported to factories/mills for processing. In Gujarat, the main factories are located at Palampur, Sidhpur Unjha and Kakosi. The seeds are passed through sieving pans to separate out dust particles, and shrivelled and deformed seed. The remaining seed is passed through grinding mills. The ground seed material is again sieved and winnowed to separate out the husk from coarse remains of the kernel. As a consequence, two products are recovered. These are called 'Kalai' and 'Gola' or 'Goli' in Gujarati. The husk-seed ratio by weight is 25:75.

'Kalai' represents parts of outer seed coating which is white, membranous and commercially most valuable. It represents isabgol of commerce; the best grade isabgol is 70 mesh.

'Gola/Goli' represents kernel or husk-free seed. It is deep red in colour, and is used as cattle feed. Two other by products, namely Khako and Lali are also used as cattle feed. Lali comprises pieces of broken embryos and other seed parts, whereas Khako as the very name suggests, constituents dirty remains of seed husk (Modi *et al.*, 1974).

Chemical Constituents and Characteristics of the Seed

Seeds of *P. ovata* contain mucilage, fatty oil, large quantities of albuminous matter, a pharmacologically inactive glucoside, namely aucubin ($C_{13}H_{19}O_8H_2O$) (Chopra, 1930; Khorana, Prabhu and Rao, 1958) and a plantiose sugar (French, 1953). Cold water extract of seeds contains polysaccharides composed of 20% uronic anhydride, 52% pentosan and 18% methyl pentosan (Laidlaw and Percival, 1949). Hot water extract of seeds contains polysaccharides, PI and II. PI yields on hydrolysis, D-xylose, L-arabinose and an aldobiuronic acid called 2D-galacturonoside-L- rhamnose and an insoluble residue. PII yields L-arabinose, D-xylose and an insoluble residue. The seed oil contains two oxygenated fatty acids, namely 9-hydoxyactadec-cis-12-enoic acid and 9-oxoactadec- cis-12-enoic acid (Jamal *et al.*, 1987).

Seeds of psyllium also contain 17-19% proteins (Patel *et al.*, 1979). The aminoacids separated from psyllium seed by paper chromatography include valine, alanine, glycine, glutamic acid, cystine, lysine, leucine, tyrosine and serine

With mild alkali treatment, the seed husk yields 85% polysaccharide (single species) and 15% of non-polysaccharides. The mucilage contained in psyllium seeds is an arabinosyl (galactosyluronic acid) rhamnosyxylan (Sandu *et al.*, 1981). The sugar and mucilage content of seed bear inverse relation at different stages of seed development (Chinoy *et al.*, 1978). To begin with, the sugar content of seed is high and the mucilage content low Reverse is true during later stages

The seed husk displays negative thixotropy when it is dispersed in water but when heated to 60°C it is transformed into a thixotropic gel with bulges and spurs, the mucilage has emulsifying properties (Bhunvara and Khorana, 1950; Mithal and Kasid, 1964, 1965) and is a good suspension agent (Mithal and Gupta, 1965) The 2% w/v mucilage of seed husk powder in cold water compares well, whereas 1.5% w/v mucilage in hot water is superior to 10% w/v starch mucilage, in binding properties (Mithal and Bhutiani, 1969). It is comparable to methyl cellulose and is superior to sodium alginate and sodium carboxymethyl cellulose in suspending properties (Mital and Gupta, 1965).

The carboxymethyl derivatives of psyllium husk is a fibrous mass, light in odour and mucilaginous in taste. It swells readily in water forming viscous mucilage of *p*H 6.7 and swelling factor 90. It is superior to isabgol husk in viscosity (Khasgiwal and Mithal, 1975), spreadability, film forming characteristics and smooth texture (Jain and Mithal, 1976). The greyish aqueous extract of husk is an excellent thickening agent.

Utility

Psyllium seed is in use in medicine since long. The Persian physician, Alhervi, prescribed its use as early as 10th century for treatment of chronic dysentery and intestinal fluxes. The seed husk of psyllium has the property of absorbing and retaining water which accounts for its utility in checking diarrhoea. In patients suffering chronic dysentery, the ulcerated surface of intestinal mucosa is soothed by the demulcent action of the mucilage; the mucilage spreads along the inner wall of intestine and protects it against the irritants present in food. Moreover, the mucilage absorbs toxins from the gut and helps in excreting them from the body. Use of 2 or 3 heaped desert spoonfuls of seed, twice a day, for a couple of months resolves all symptoms of chronic dysentery and eliminates *Entamoeba histolytica*, if present, from the patients body. The mucilage of seed husk acts as lubricant in the large intestine. The husk is consumed alone or is mixed with different chemicals, such as powdered anhydrous dextrose, sodium bicarbonate, citric acid etc. (Virmani *et al.*, 1980).

The Central Drug Research Institute, Lucknow, has developed sweet, palatable and variously flavoured granules and fine powder of isabgol, that is marketted as Ligafin and is prescribed for use as laxative. Some American Pharmaceutical companies, like M/s Searle Laboratories, M/s Parke Davis, M/s J.B. Williams etc. supplement the husk with fruit essence and sell it under various brand names such as 'Metamucil', 'Siblin', 'Seraton' etc.

Isabgol is diuretic, it alleviates kidney and bladder complaints, gonorrhea, urethritis and hemorrhoids. It removes burning sensation in feet, relieves polyuria, difficult micturition and tones up bladder. It is also effective in checking spermatorea. It is recommended for use by pregnant women. The CDRI, Lucknow has developed Isaptent sticks from isabgol for use in termination of pregnancy. These are marketted under the trade name, "Isabgol (dilex-C) tent". According to Khanna *et al.*, (1980) Isaptent sticks are superior to Laminariatent.

Seeds of *P. ovata* soaked in water are recommended for treatment of cancer (Ahmad, Farooqui and Siddiqui, 1985; Hartwell, 1970). The seed husk is also effective in reducing serum-cholesterol level. The oil contained in Isabgol embryo is rich in linoleic acid and has the potentiality as dietary hypocholesterolemic agent. The oil content in embryo and endosperm is 14.7 and 8.8% respectively (Atal, Kapur and Siddiqui, 1964). Siddiqui, Kapur and Atal (1964) hold that Isabgol oil

is more potent than safflower oil for reducing serum-cholesterol level. The efficacy is increased when the husk is consumed with metronidazole (Ikram, 1984). Ingestion of 10 g isabgol a day for a month reduces serum cholesterol level by 9.6% and triglyceride by 8.6% (Goswami, 1988).

Decoction of *P. ovata* mixed with honey is good for treatment of sore throat and bronchitis. The liquid obtained after boiling psyllium seed is chilled and used as eye drops. It is also used to get rid of pimples and check hair fall. Regular use of isabgol makes the skin the removes all kinds of blemishes.

Seed husk of isabgol is also used in ice-cream industry as substitute for sodium alginate (Upadhyay, Patel and Vyas, 1978). The mucilage is used as an ingredient in chocolate making, textile sizing, manufacturing cosmetics (Singh and Virmani, 1982) and setting and dressing hair (Roia, 1966). The seeds and husk are also used in dyeing. Psyllium is also a source of commercial gum (Farooqi, 1976).

Tribals use isabgol for several purposes; the Santhals use it to relieve pain and treat bronchitis (Jain and Tarafder, 1970). The tribal inhabitants of North Gujarat consume seed decoction of psyllium as a cooling demulcent to cure diarrhoea and dysentery.

As pointed out before also 'Isabgol-Gola' - a byproduct of psyllium is used as cattle feed (Shukla *et al.*, 1983). Its consumption has no adverse effect on production and composition of milk and the body weight of milch cows (Desai *et al.*, 1980).

Toxicity

Use of psyllium as laxative does not induce any side effects. However, the plants cause allergy in the work environment (Husain, 1977; Machado, Zetterstom and Fagerberg, 1979). Occasional asthma is reported among people who work with psyllium (Bernton, 1969; Busse and Schoenwetter, 1975). Unsoaked seeds may cause gastrointestinal irritation, inflammation, mechanical obstruction and constipation whereas powdered or chewed seeds release a pigment which is injurious to kidneys (Singh and Virmani, 1982).

Improvement

Though India holds monopoly in the world trade of isabgol, it fails to meet the global demand. Therefore, efforts are a foot to increase seed yield using conventional and non-conventional techniques in light of its breeding and genetic systems.

Selection: In view of lack of in-built variation in the crop, the prospects of breeding high yielding varieties through selection are limited. Yet, Kantikar and Pendse (1961) hold that cultivars from Palampur (Banaskantha) are superior to those from Mehsana, Visnagar and Sidhpur. Chandra (1967) rates the strains grown in Patan (Mehsana) superior to others. A high yielding strain, Gujarat Isabgol-1 has been

bred at Pilwai Research Station of GAU, Anand. It yields 10-12% more seed than local cultivars and is, therefore, popular among farmers. Another high yielding strain, HI-5, has been bred at Hissar (Punia, Sharma and Verma, 1985). Single plant selections exercised within the Italian strain, EC42706, which is superior to local strains in synchronous maturity, compact spike and dwarf plant size have led to establishment of some promising genotypes, designated EC42706-1, 1-4-, EC42706-2/P-2 and EC42706-B (Mital and Singh, 1986).

Hybridization: Pilger (1937) has referred *P. ovata* along with some old world allies, namely *P. albicans, P. boisseri, P. cylindrica* and *P. stocksii* to section Leucopysllium of the genus *Plantago*. Crossability relations of these species with *P. ovata* have not been established so far.

Interspecific hybridization is difficult to achieve in genus *Plantago*. Difference in chromosome number at species level is one of the impediments. Only three species, namely *P. fastigata, P. insularis and P. ovata*, are based on 4 (Fedorov, 1969). Stebbins and Day (1967) have successfully hybridized *P. ovata* (n=4) with *P. insularis* (n=4), but the hybrid was of little consequence on account of its low fertility. Punia, Sharma & Verma (1985) claim to have crossed *P. ovata* (n=4) with *P. lanceolata* (n=6) but details of the hybrid raised are wanting.

In vitro Studies: As a first step towards exploiting tissue culture technique for improving psyllium, the technique for regenerating plants from shoot apices and hypocotyl explants *in vitro* has been standardised. (Wakhlu and Barna, 1988, 1989; Barna and Wakhlu, 1988, 1989). The plants raised *in vitro* have been transferred to field on a small scale. Despite the extensive variation in chromosome number of callus cells (from 8 to 48), the regenerated plants conform to seed grown plants (Wakhlu and Barna, 1989) with respect to chromosome count and karyotype.

Induced Polyploidy: Induced polyploidy has been tried in *P. ovata* with the aim of harvesting such advantages as are associated with polyploidy. Tetraploids have been raised by Chandler (1954), Mital *et al.*, (1975), Zadoo and Farooqi (1977) and Bhagat *et al.*, (1980). The tetraploid plants are shorter, sturdier and non- lodging. Their demerit lies in low seed output. In a crop like psyllium, where seed is the commercially important part, sterility is a serious handicap. Comparison of C_2 plants with their diploid counterparts has revealed that polyploidy shoots up the number of tillers and spikes per plant by 15.4 %. Concomitantly the number of flowers and seeds per spike is reduced by 26.5 and 40.17% respectively. As a consequence, seed output per plant is reduced by 29.29%. The loss is made good by the increase, the tetraploid seeds register in weight (51.91 %) and volume (50 %). Additionally, the tetraploid is superior with respect to seed mucilage characters. In C_4 generation and thereafter, the tetraploid plants lose the edge they have over diploids with regard to number of tillers and spikes per plant. This reduction deteriorates seed set further in later generations and neutralises the advantages conferred by polyploidy. These handicaps notwithstanding, one can hope that by

exercising selection for higher tiller and spike count per plant, a few genotypes can be selected which are superior in seed yield.

Induced Mutations: In order to overcome the problem of lack of variability in *P. ovata*, mutations have been tried to induce variation. Physical as well as chemical mutagens have been used. Mital and others hold that the populations derived from NMU treatment have high frequency of favourable genotypes (Bhagat, Maheshwari and Hardas, 1980; Mital, Bhagat and Maheshwari, 1975). However, Sareen and Koul (1991, 1992) find that psyllium seeds are relatively resistant to radiations. Lower doses of gamma rays like 20 KR treatment induced increase in seed yield per plant.

Wild Allies

North-Western Himalayas host a number of wild allies of *P.ovata, namely P.depressa, P.amplexicaulis, P.exigua, P.indica, P.himalaica, P.lagopus, P.lanceolata and P. major*. While most of these are weeds of the temperate regions, a few grow in the dry, sandy soils of the subtropics. These species differ from *P.ovata* in chromosome number, details of chromosome complement, plant morphology and their tolerance to climatic extremes. Hybridization of *P. ovata* with these taxa is desirable for purposes of transferring to it desirable traits such as large seed size from *P. amplexicaulis*, high swelling factor from *P. psyllium*, non- shattering spikes from *P. depressa, P.exigua* and *P.major* and drought and frost resistance from *P. lanceolata* and *P. major*. Should the genes controlling these traits be transferred to *P. ovata*, it will mean a leap forward in achieving the goal of improving psyllium

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Indian Ginseng — Its Present Status and Future Perspectives

R.S. Kapil, Y.K. Sarin, R. Kapoor, Y.S. Bedi* & S.N. Sharma

Regional Research Laboratory, Jammu - 180 001

Introduction

GINSENG belongs to genus *Panax* (Araliaceae), has been recognized for centuries as an important plant used in oriental medicines. The name *Panax* is derived from Greek words "Pan" meaning 'all' and "axos" meaning 'cure' to mean Panacea or all curing and its properties have been no less touted. It is perhaps the most widely recognized plant used in traditional medicine and now plays a major role in the ever increasing herbal health care market of the world. Eight species are ascribed to this genus (Airy Shaw, 1973), out of these, the roots and rhizomes of *P. ginseng, P japonicum, P. pseudoginseng* and its varieties namely, var. *notoginseng* and var. *major* which are native to Asia and *P. quinquefolium* and *P. trifolium*, native to N. America are used medicinally (Nair, 1991).

Earlier it was used in the treatment of asthenia, atherosclerosis, blood and bleeding disorders, colitis and to relieve symptoms of aging, cancer and senility. Now evidence has emerged that root possesses a general strengthening effect, raises mental and physical capacity, exerts a protectant effect against experimental diabetes, neurosis, radiation sickness and some cancers. Moreover, with the discovery of adaptogenic effect of its saponin content, (Anonymous, 1990) its popularity has increased many folds. It is a popular ingredient in herbal teas, shampoos and cosmetics, and its extract, capsules or tablets are widely used as medicine. Interestingly, all the products of ginseng are not usually associated with any serious adverse reactions (Anonymous l.c.).

Components and Importance of Indian Ginseng

Panax ginseng, P. quinquefolium and *P. japonicus* are the main sources of Korean, American and Japanese ginseng, respectively. Whereas *P. pseudoginseng* provides chinese wonder drug "Sanchi" used to cure all diseases, even cancer. Among these, Korean ginseng is widely used and more accepted in pharmaceutical industry. At present Korean ginseng worth more than 5 million rupees is being annually imported by India. However, Himalayan species of *Panax* are often referred as Indian ginseng. It is least understood of all ginsengs and is a conglomerate of 5 different species of *Panax* namely, *P. pseudoginseng, P. sikkimensis, P. bipinnatifidus, P. burkillianus* and *P. schinseng* (Joshi *et al.*, 1991, Changkija and Kumar, 1992, Mehta and Haridasan, 1993). The importance of Indian ginseng to pharmaceutical industry has increased many fold after the recent studies by Dua *et al.*, (1989), who have found it to be comparable and in some tests even better than Korean ginseng. Also Shukla and Thakur (1987) have established that almost all the active saponins of Korean ginseng are present in Indian pseudoginseng. These reports have generated a lot of scientific and commercial interest in Indian ginseng, which has resulted in a blatant exploitation of its roots and rhizomes from natural habitats. This necessitates a need for the development of strategies for getting the sustainable supply of its roots by bringing it under cultivation. This will not only help us in protecting rich germplasm of Indian ginseng, but will also help in saving the valuable foreign exchange being spent in importing Korean ginseng.

Keeping this in mind, studies were initiated in 1990 on Indian ginseng. Surveys were conducted in N.E.states of Meghalaya, Arunachal Pradesh and Nagaland to record the availability of germplasm, study its natural habitat and biology and to initiate work on its domestication in the experimental fields of Regional Research Laboratory, Extension Centre, Palampur.

In the following pages, botany, distribution, utilization and cultivation efforts on Indian ginseng are presented. Necessity to conserve Indian species of *Panax* has also been emphasised.

Botany

Taxonomy of *Panax* species continues to be controversial. There is a lot of confusion about the number of *Panax* species falling under Indian ginseng. Hara (1966) attributes all the ginseng collections from Eastern Himalayas to only one species, namely, *P. pseudoginseng* Wall. subsp. *himalicus* Hara and its two varieties viz. var. *angustifolius* (Burkill) Li and var. *bipinnatifida* (Seem) Li. Later Banerjee (1968) recognised 4 species (*P. fruticosus* L., *P. pseudoginseng* Wall., *P. sikkimensis* Ban. and *P. assamicus* Ban.) and one variety (*P. pseudoginseng* var. *bipinnatifida* (Seem) Li from India. All these except *P. fruticosus* are associated with the name Indian ginseng and are growing in E. Himalayas. Cannon (1979) treated *P. sikkimensis* and *P. assamicus* as synonyms of *P. pseudoginseng* var. *angustifo-*

lius (Burkill) Li. Whereas, Bennet and Sharma (1983) on the basis of leaf and rhizome morphology treated both *P. bipinnatifidus* Seem. and *P. sikkimensis*, specifically distinct from *P. pseudoginseng* and attributed atleast these 3 species to Indian ginseng. Bennet and Viswanathan (1984), on the basis of their field studies from Shillong found that the most commonly available ginseng plants, on the basis of rhizome and leaf characters are specifically distinct from *P. pseudoginseng* var. *angustifolius* of Li (1942) should be raised to specific rank as *P. burkillianus* Benn. & Visw.

With the record of *P. schinseng* Nees. (*P. ginseng* C.A. Meyer) from Nagaland by Changkija and Kumar (1992) and from our field studies in north eastern states, we believe that the Indian ginseng is a conglomerate of 5 species of *Panax* namely, *P. sikkimensis* Ban., *P. burkillianus* Benn. & Visw., *P. bipinnatifidus* Seem., *P. schinseng* Nees. and *P. pseudoginseng* Wall.

The Indian ginsengs are small perennial deciduous herbs. The aerial vegetative shoot of mature plant is 25-150 cm tall and is composed of single hollow, weak, green to reddish green, glabrous erect stem, which is arising subapically from rhizome and terminating in whorl of 3-10 palmately compound leaves. The growth of aerial shoots is determinant. A single shoot primordium is differentiated subapically on a sub-terranean rhizome, the year prior to expression and the growth of aerial shoot in any year is limited to the expansion of the preformed primordium. The vegetative growth and flowering takes place during May to June and the fruits ripen during October. The rhizome with root system is underground and is variable morphologically in different *Panax* species (Table 1). The flowers are creamy yellow to greenish white and hermaphrodite, styles 3, free or united at the base. Fruits small, globose berries with 1-3 seeds.

There is difference of opinion regarding the colour of mature fruits. Earlier authors, depending upon their time of collection have given colour of fruits as crimson red (Changkija and Kumar, 1992) in *P. schinseng*, red or half red, half black (Clarke, 1879), red (Wallich, 1831) in *P. pseudoginseng*, lower 2/3 red and upper 1/3 black (Burkill, 1902) in *P. bipinnatifidus*, red and black (Bennet and Sharma, 1983) in *P. sikkimensis* and crimson red (Bennet and Viswanathan, 1984) in *P. burkillianus*. During field surveys by the present authors, it was observed that in all the *Panax* species falling under Indian ginseng ripe fruits are initially red and then berries gradually start turning black from upper end. So depending upon maturity, one can find fully red or both black and red berries. Thus we disagree with Bennet and Sharma (1983) in considering colour of fruit in genus *Panax* of any taxonomic significance.

Distribution

International range of Indian ginseng is in the interior temperate mountainous regions of north east India, northern Burma, Bhutan, Nepal, China and Southern

Table 1 — Distinguishing features of 5 Indian *Panax* species

S. No.	Characters	*P. pseudoginseng*	P. burkillianus	*P. schinseng*	*P. sikkimensis*	*P. bipinnatifidus*
1.	Rhizome and root stock	Tuber fasicled, fusiform, hard, base of stem swollen with fleshy deciduous scales	Creeping, horizontal ginger like with conspicuous annual abscission scars	Short, mortar shaped attached to stout roots which are less branched and thick	Single, horizontal knotted with ring like persistent scales with a globose tuber at distal end	Same as *P. sikkimensis*
2.	Leaves	3-5 foliate	5-7 foliate	5-6 foliate	5-7 foliate	3-5 foliate
3.	Leaflets	Lancaeolate to oblong lanceolate, serrate, acuminate	Narrow lanceolate, serrate, caudate	Ovate or obovate, dentate, acuminate	Oblong lanceolate serrate, acuminate	Lanceolate, bippinatifid, lobes, serrated, acuminated

Tibet. In India, it is predominantly Himalayan in distribution. It extends from Uttar Pradesh (W. Himalayas) to Arunachal Pradesh (E. Himalayas) between altitudes of 1500-3000 m. Details of distribution sites (latitude 23°80′ - 29°70′ N; longitude 79°50′ - 98°2′ E) is given in Map 1. Its distribution is restricted to the areas with annual rainfall of 800 to 2500 mm, temperature range of 4 to 25°C and soil pH of 5- 6.5. It thrives well in localities with well drained moist forest soil, rich in organic matter and leaf litter, growing under temperate forest cover with thick and natural vegetation, imparting 50 to 75% shade. Locality wise distribution of Indian ginseng is given in Table 2.

It is believed that if not all, most of the ginseng from Arunachal Pradesh and Sikkim belong to *P. sikkimensis* and *P. bipinnatifidus* and those from Meghalaya, Darjeeling and Nagaland belong to *P. burkillianus* and *P. pseudoginseng* while there is also a single report each of *P. schinseng* from Nagaland and *P. pseudoginseng* from Uttar Pradesh.

Chemical and Pharmacological Status

Details of chemistry of Indian ginseng is beyond the perview of this article. However, a cursory comparison of chemical status of Indian ginseng with Korean ginseng is taken up.

Keeping in view the medicinal importance of ginseng and lack of chemical studies on Indian ginseng, a systematic attempt was made by CIMAP, Lucknow scientists to study its chemistry on their collection from Darjeeling (W. Bengal). It leads to the discovery of lipids from rhizomes (Shukla and Thakur, 1985, 1986) and leaves (Shukla and Thakur, 1989). Also it has been found to be a rich source of oleanolic acid saponins while dammarane saponins are present in minor amounts (Shukla and Thakur, 1986). From rhizomes of Indian ginseng, ginsenosides Rb_1, -Rb_2, -Rb_3, -Rc, -Rd, -Re, -Rg_1 and -Ro; pseudoginsenosides, F_{11}, RI_1, RI_2, RP_1 and RT_1 ; and chikusetusaponins IVa and IV (Shukla and Thakur, 1986, 1987, 1988 a,b, 1990 a, Shukla, 1989) are isolated, while the leaves (Shukla and Thakur, 1990 b) yielded all these saponins, except for pseudoginsenosides RI_1 and RI_2 which were present only in the rhizomes of *P.burkillianus* (=*P.pseudoginseng* var. *angustifolius*). It is interesting to mention that the active saponins of Korean ginseng like, -Rb_1, -Rb_2, -Rd, -Re, -Rg_1 and Ro and chikusetsusaponin VI are also present in Indian ginseng (Tanaka and Kasai, 1984, Shukla and Thakur, 1988 b and Anonymous, 1990).

Diverse activities are attributed to the roots of ginseng which range from CNS depression or stimulation, variable effects on systemic blood pressure, analgesic and antiinflammatory protection against the stress induced ulcers, and adaptogenic activity (Anonymous 1990). Dua *et al.*, (1989) compared the crude extract and saponins of Indian ginseng for adaptogenic, antiinflammatory and immunostimulant activities using a battery of pharmacological tests and compared with Korean

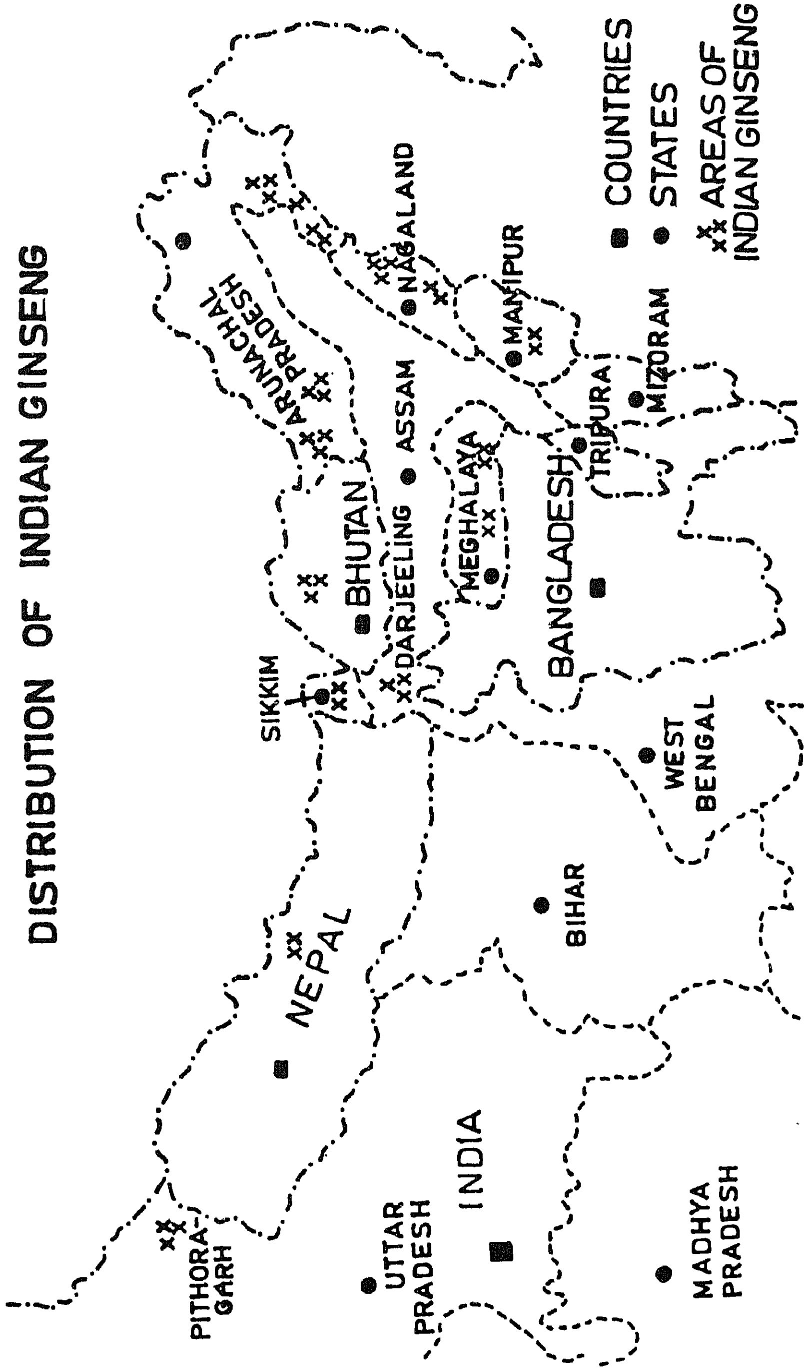
DISTRIBUTION OF INDIAN GINSENG
■ COUNTRIES
● STATES
xx AREAS OF INDIAN GINSENG
ARUNACHAL PRADESH
NAGALAND
MANIPUR
MIZORAM
TRIPURA
ASSAM
MEGHALAYA
BANGLADESH
BHUTAN
DARJEELING
SIKKIM
WEST BENGAL
BIHAR
NEPAL
INDIA
UTTAR PRADESH
PITHORA-GARH
MADHYA PRADESH

Table 2 — Distribution of Indian ginseng

S. No.	State	District	Locality with altitude*	
1.	Uttar Pradesh	Pithoragarh	Thala Ruigair,	3,300 m Narayan
			Ashram-Sridhang areas,	1950-2,700 m
2.	Arunachal Pradesh	Twang	Twang,	2,800-3,400 m
		Lower Subansiri	Tale Valley,	2,400 m
		Lohit	Simbi Hot spring }	1,600 - 2,700m
		Tirap	Rahu-Walkka }	
3	Meghalaya	Shillong	Upper Shillong,	1,830 m
			Shillong Peak,	1,950 m
			Mauphlang,	1,830 m
			Nongstoin,	1,830 m
4.	Manipur	–	Shirohee,	2,135 m
5.	West Bengal	Darjeeling	Kagjhora,	2,135 m
			Singalila Range,	2,700 m
6.	Nagaland	Tuensang	Patkoi Range,	1,800-2,500m
7.	Sikkim	North District	Lachung,	2,593 m
			Zemu Valley,	2,745 m
			Sonada,	1,982 m
			Sundukphoo,	3,050 m
			Neebi to Bukeem,	2,135-2,440 m
			Laghep,	3,050 m
			Mount Singolah,	3,660 m
			Mount Tankre,	3,507 m

* Based on Joshi *et al.*, (1986) Mehta and Haridasan (1993) Herbarium collections at CNH, Calcutta, FRI, Dehradun and present studies

ginseng saponins. They found that the swimming performance of rats markedly increased with Indian ginseng than the Korean ginseng saponins. However, gastric ulceration induced by swimming stress as well as immobilization stress was inhibited equally by both types of saponins. Crude Indian ginseng as well as its saponins had weak anti-inflammatory activity in acute as well as subacute models of inflammation. As immunostimulant, saponins from Indian ginseng were found to be more effective than the saponins of the Korean ginseng.

Since the earlier chemical and pharmacological studies on Indian ginseng are confined only to Darjeeling area, it is suggested that such studies are to be extended to the collection from other areas of the himalayas, for proper evaluation of

germplasm of Indian ginseng, so that strains with higher biomass yield as well as higher activity be selected for bringing under cultivation and future breeding programmes.

Utilization and Conservation

Ginseng is the most precious drug with cure all properties (Joshi *et al.*, 1991). Unfortunately due to lack of proper understanding and knowledge, there has been no serious and consistent organised scientific effort for collection of Indian ginseng, its utilization and to ascertain cultivation prospects. The short life span of aerial shoot during monsoon, is the plus point with ginseng to evade the onslaught of plant collector and also its habitat during monsoon is not easily accessible.

However, massive deforestation, specially in the states of Nagaland and Meghalaya has lead to the habitat destruction and disturbance, which inturn has adversely effected natural regeneration (Bennet and Sharma, 1983). Further, indiscriminate collection and smuggling (Changkija and Kumar, 1992) of rhizomes and root stocks of this wonder drug to China and Burma has lead to almost depletion of the species from Nagaland. In north eastern states of Maghalaya, Arunachal Pradesh and Nagaland, now natural populations of Indian ginseng are only available in either sacred grooves or inaccessible remote regions of these states. Like *P. quinquefolium*, where in natural populations death rate is 14 times higher than regeneration rate (Hu *et al.*, 1980), Indian ginseng is also being depleted at an alarming rate due to higher death rate. But keeping in view the great genetic diversity of species of *Panax* falling under Indian ginseng (Nair, 1991), it is advisable to protect this rich germplasm for future breeding programmes, of incorporating desirable characteristics to already commercialized American and Korean ginsengs.

Thus to protect the surviving natural populations and to increase the productivity, an augmentative plantation and aided natural regeneration are the only answer (Mehta and Haridasan, 1993). So far only Joshi *et al.*, (1991) have done some preliminary cultivation trials of Indian ginseng under laboratory conditions, but as such our knowledge is fragmentary.

Cultivation

Based on our own experience on cultivation trials of Indian ginseng at Palampur (Himachal Pradesh) from rhizomes and also available information (Joshi *et al.*, 1991 and Mehta and Haridasan, 1993), cultivation details of Indian ginseng are discussed as under:

Climate and Soil : Ginseng has typical climatic and soil requirements for its proper growth. It can be profitably cultivated in temperate areas, with temperature range of 4-25°C, soil *p*H of 5-6.5 and annual rainfall of 800-2,500 mm. It prefers well

drained, peaty, sandy loam soil with a thick cover of humus, derived from broad leaf species and rich in potassium.

Collection of Planting Material: Crop of ginseng can be raised either from seeds or rhizomes. Fruits mature in the month of September and October. After collection, the seeds are extracted from fruits, washed and then shade dried for proper storage, and are sown on the onset of winters next year. During this period the full embryonic development of seed is achieved.

Rhizomes can also be collected during September-October, but are sown the same year. From our field observations, it has been found that due to difference in rhizome morphology, *P. bipinnatifidus* and *P. sikkimensis* produce lesser biomass of rhizomes (3-5 gm/plant), whereas it is higher in *P. schinseng, P.pseudoginseng* and *P.burkillianus* (20-30 gms/plant) Thus it seems probable that the latter three species should be brought under cultivation for higher yield, but such inference is yet to be supported by chemical/pharmacological studies.

Raising of Nursery

(i) From Seeds: Before sowing, stratification of seeds is required due to hard seed coat. For stratification, healthy seeds are mixed with fine sand in 1:5 ratio (Joshi *et al.*, 1991) for about three months. Selected seeds with partially opened seed coats are sown in prepared raised nursery beds, prepared in land which is lying fallow for atleast two years, after turning soil to greater depths for atleast fifteen times (Joshi *et al.*, l.c.). To improve soil texture; sand, leaf and grass mould is added. It has been observed that mixing of ash from leaves and grasses also adds to the quality of soil. For healthy and even growth of seedlings line sowing is recommended. Seeds germinate in April next, and continue to grow till early October, when the aerial parts wither off. At this stage mulching of nursery beds is required to avoid frost damage to underground parts. In next March-April the seedlings are dug out and transplanted in the fields.

(ii) From Rhizomes : Nursery plants can also be raised from rhizomes by splitting them into smaller segments with atleast two nodes. Thus from a single healthy rhizome, 10-20 plantlets can be raised.

Rhizome segments are pretreated with 0.3% bavistin to check any attack of fungal organism and are sown in nursery beds at 10 ×10 cm distance in the month of November. Plantlets appear by the end of April next. With this method, during first year sprouting percentage of 60-70% is achieved. Sprouted plants are transplanted to field, same year whereas, unsprouted, healthy rhizome segments are left in nursery beds for sprouting in the next season. During winters all precautions are taken to protect them from frost.

Transplantation

Healthy seedlings/plantlets are sorted for transplantation in the already prepared fields with overhead shade, to maintain 8 to 12% of full sunlight in the field. Plantation is done at 30×30 cm spacing with about 10 cm soil humus cover. Field is watered after transplantation operation.

Management of Crop

Weeding and irrigation operations are carried out as and when required. As the field is rich in organic matter, so the external application of chemical fertilizers is not recommended. Hong (1982) is of the view that in Korean ginseng also application of chemical fertilizers adversely affects the quality of ginseng.

Depending upon the intensity of sun and season, shade is also increased or decreased. For good sprouting and to ensure aeration, in the subsequent years, it is necessary to loosen the bed soil and also to apply leaf mould as top dressing every year. Also the fallen bed sides are repaired with the furrow soil. During winter months the root stock is protected from frost by grass mulch. Harvesting of the crop is usually carried out in sixth year of plantation (Joshi *et al.*, 1991) in the months of September - October.

Diseases

It is reported that *Alternaria* blight and other fungal diseases discouraged many growers to cultivate ginseng in America (Graham, 1966). Indian ginseng is also highly susceptible to rhizome rot caused by *Fusarium* during rainy season, which causes an extensive damage to the crop. Also seedling damping off is observed at nursery stage which is caused by *Rhizoctonia*. In addition it is also attacked by rust (*Uromyces* sp.) and downy mildew. Thrumalachar and Chupp (1948) recorded leaf spot of ginseng from a natural population, caused by *Cercospora panacis*.

Rhizome rot and seedling damping off is controlled by drenching of ginseng field with 0.1% bavistin.

Conclusion

Perusal of literature reveal that despite the rich potential of promoting ginseng as a cash crop in himalayan region, not much has been done in this direction, mainly due to lack of knowledge and information about this plant. The present communication is a modest step towards this end. However there is a need to develop agrotechnology for this crop, for which studies are required to be initiated to generate information on the soil and microclimatic requirements of this plant, its genetic improvement, mass propagation, disease management, harvest and storage technology. In addition chemical and pharmacological studies are required to be undertaken on all the *Panax* species referred to Indian ginseng after bringing them

under cultivation under uniform environmental conditions, to evaluate their comparative efficacy, so as to select the most potential species for general cultivation. This will not only help us in saving valuable foreign exchange but will also help in boosting the economic status of farmers from temperate Himalayas.

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Rasaayana Drugs[1]

S. S. Handa

Regional Research Laboratory, Canal Road, Jammu - 180 001

IT was well known to Ayurvedic physicians that the delicate cellular machinery of the body suffers from trauma (stress) resulting in wear and tear of different body structures and deterioration of the functional capacity of human being. For this, procedures of revitalization and rejuvenation were adopted to increase the power of resistance to disease (increased immunity) and these procedures retarded advancement of aging also (Charaksutrasthana, Chapter 7, pp 46-50). Rasaayanas were prescribed for a particular period and strict regiments were observed regarding diet. Thus, vitalizers which appear a kin to antistress agents of plant origin, whether from Ayurveda (2700 - 600 B.C.), Chinese or Russian traditional medicines (*Panax ginseng* and *Eleutherococcus senticosus*) may be acting as inducers of enzyme systems like interferon (for fighting viral diseases) and of Succinate Dehydrogenase (SDH) enzyme responsible for the conservation and utilization of energy particularly during stress. Ayurveda was fully alive to the concept of vitalization therapy and need to keep a disease-free, healthy life in its totality of both physical and mental well-being (sound health).

The following Sutra illustrates the facts about the usefulness of vitalisation therapy.

The verse says "long life, increased memory and intelligence, freedom from disease, youth, excellence of lustre, complexion, voice, strength of body and mind are obtained by vitalizers". These materials termed as "Rasaayana" may be inducers

1Paper reproduced from Pharmatimes (Dec.,93 and March,94).

of enzymes, hormones etc. which the body needs for adaptation and survival during health, stress and disease.

Sushruta (Sutra 1/15) defining Rasaayana therapy says that this therapy arrests ageing (Vaya-sthapan), increases life span (Ayushkarm),intelligence (Medha), and strength (Bala) and enables one to prevent disease (Rogapharana samartham). According to Sharangdhara (Purva 4/41) Rasaayana drugs keep off ageing (Jara) and prevent disease (Vyadhi). Some such drugs mentioned by Charaka include (Charaka Sam. Sutra 4/1, 2,7,50):

Bala	*Sida cordifolia*
Atibala	*Abutilon indicum*
Guduchi	*Tinospora cordifolia*
Vacha	*Acorus calamus*
Tulsi	*Ocimum sanctum*
Ashvagandha	*Withania somnifera*
Amalaki	*Emblica officinalis*
Shatavari	*Asparagus racemosus*
Pippali	*Piper longum*
Guggulu	*Commiphora mukul*
Bhallataka	*Semecarpus anacardium*
Mandookaparni	*Centella asiatica*

However, Table 1 indicates all such drugs classified into Medhya and Rasaayana (Satyavati, 1993). Multifactorial actions of Rasaayana are given in figure 1.

Much before the concept of immunity or microbes was known, a large number of plants were used in the traditional medicine of Europe, China and India for rejuvenation therapy and treatment of chronic ailments (Simons *et al.*, 1989; Atal *et al.*, 1986).

Immunomodulatory substances of varying chemical structures and molecular sizes exist in these plants, which exert that effect by modulating several facts of the immune system such as activation of macrophages for enhanced generation, oxygen radical and stimulation of cytotoxic activity and phagocytosis, proliferation of lymphocytes leading to production and also cytotoxic induction of T-helper and natural killer (NK) cells and activation of complement pathways. Immunostimulants offer promise in enhancing antigen specific (vaccines) and non-specific immune response against infection and malignancy, immuno therapy of viral infection and possibly cancer and potentiating the efficacy of drugs in immuno compromised host. Much more could have been achieved by Indian scientists in the field of Rasaayana therapy. Only a few plants out of the entire list of Medhya and Rasaayana drugs have been worked at a rudimentary level and some such plants have been mentioned in this presentation.

Fig. 1 Multifactorial Action of Rasaayana

However, much more coordinated efforts of Ayurvedic, Allopathic clinicians, pharmacologists, pharmacognosists and phytochemists are needed before we proceed towards achieving something in the field of Rasaayana therapy. On the other hand Chinese plants *Ginkgo biloba* and *Panax ginseng* have been extensively investigated. Brief account of these two plants has been given at the end. The notion of "resistance" to disease and the idea that such resistance can be modified by life experience and by emotional states, forms one of the basic tenets of Ayurveda, the ancient Indian system of medicine, thus avoiding the Cartesian dichotomization of mind and body. *Withania somnifera* Dun (Family

Solanaceae) is one of the plants used in Ayurveda to prevent or treat disease through the restoration of a healthy balance of life forces and an integrated relationship to the environment (Sharma, 1978). Brekhman and Dardymov (1969) have shown that certain plant extracts possess significant anti-stress activity in animals and in man. The Indian indigenous drug, 'Asvagandha' (*Withania somnifera*) is used as 'Medhya Rasaayana' (for the treatment of mental diseases and anxiety states) in Ayurveda. Clinical trials of the drug in patients of anxiety and improvement of mental functions (Malviya, 1976; Singh and Malviya, 1978). The drug reduces the level of acetylcholine and catecholamines and increases the level of serotonin and histamine in brain tissues. The rise in 5-hydroxytryptamine level may be a compensatory response to the depletion of catecholamines. The total methanol : water (1:1) extract of the roots of *W. somnifera* and equimolar combinations of two acylsteryl glucosides sitoindoside VII and VIII and witheferin-A attenuated stress induced responses from anxiety, depression, analgesia thermic changes, gastric ulcers and convulsions. Stress induced depletion of adrenal ascorbic acid and corticosterone were restored causing adrenocortical activation. Human and rat urine contain an endogenous MAO inhibitor, which has been named tribulin and acts on both MAO-A and MAO-B. Tribulin output is related to stress and is augmented during stress. Like other anxiolytics, *W. somnifera* has been found to reduce this augmented tribulin (Bhattacharya *et al.*, 1987). There are four chemotypes of *W. somnifera* so far recognised within the natural population of this plant. The main constituent of chemotype-I is witheferin-A and that of chemotype-II withanolide-D. Chemotype-III is characterised by the presence of two groups of compounds, one group with compounds possessing a normal stereochemistry at C_{17} (i.e. B-oriented side chain) such as withanolide G & H whereas in the other group the side chain-oriented (withanolide E & F). The chemotype-IV is rich in witheferin-A and withanone (Kirson *et al.*, 1977). Due to these variations biological activity varies significantly and one must ensure the right chemotype producing antistress and adaptogenic activity.

For stress related imbalances of the body, *Ocimum sanctum* has been used in various human ailments in Indian system of medicine. Hindus consider this plant as sacred and is planted in the courtyard of traditional Hindu houses for worship. For validating the claims of antistress activity, the experimental models employed include swimming endurance test, adrenal function test and tests for measuring antiulcer activity. Ascorbic acid content of adrenals was taken as index of adrenal functions. The ED_{50} values for plant extract was calculated by probit analysis for the doses administered on 7th day 1 hour before tests and mean antistress units (ASU) of activity was calculated. Considering the ASU of *O. sanctum* as 1, the relative potency of other two plant extracts was calculated. Acute-toxicity (LD_{50}) value for 3 plant extracts viz. *O. sanctum, Eleutherococcus senticosus* and *Panax ginseng* were determined in mice. From the LD_{50} and ED_{50} (ASU) value, the safety

ratio (safety margin) was obtained (Singh *et al.*, 1991). *O. sanctum* was found to possess potent antistress activity (Bhargava & Singh, 1981). It increased the survival time in swimming mice in dose dependent manner. Further it prevented stress induced (swimming) increase in adrenal weight and decrease of adrenal ascorbic acid. Percent incidence of stress (immobilisation for 18 hr.) induced gastric ulcers in albino rats was also reduced significantly. On the basis of ASU the relative potency of *O. sanctum, E.senticosus* and *P. ginseng* was calculated and found to be 1.0.83 and 0.53 respectively. The acute LD_{50} values for *O. sanctum, E.senticosus* and *P. ginseng* were found to be 4200, 3880 and 5260 mg/kg p.o. respectively and from there safety margin for each plant was calculated. *P. ginseng* has been decribed to produce corticoid like syndrome causing insomnia, oedema, hypertension and withdrawl symptoms (restlessness and diarrhoea). These side effects of ginseng limit its use as a tonic while such effects have not been observed with *O. sanctum*. Thus *O. sanctum* has a better safety margin and is a more potent antistress agent than *E senticosus* or *P. ginseng*. Beneficial effects of *O. sanctum* in viral encephalitis and stress related hypertension have been observed in clinical trials (Singh *et al.*, 1991 a). Decrease in the brain levels of adrenaline and noradrenaline occurs due to stress (Zigmond & Harvey, 1970). Pretreatment with alcoholic ext. of *O. sanctum* prevented this stress induced response. It is possible that level of adrenaline and noradrenaline decreases because of the increased utilization of these neurotransmitters in the brain tissue during stress. The normalization action of the plant extract on the level of adrenaline and noradrenaline may be helping the body to cope better during stress.

Variable opinions have been expressed about the level of brain dopamine (DA) in stress, a decrease (Blane *et al.*, 1980), an increase (Reinhardt *et al.*, 1979) and no change (Tissari *et al.*, 1979). In case of *O. sanctum* extract there was found a further increase due to stress. It appears that adrenaline and noradrenaline are utilized during stress and the DA level in the brain rises as a compensatory mechanism, as a precursor for synthesis of more of adrenaline and noradrenaline to cope with the demand. *O. sanctum* facilitates this compensatory mechanism (Singh *et al.*, 1991). The serotin level (5-HT) of brain has been reported to increase after stress (Morgom *et al.*, 1975). 5-HT level increased after stress was found significantly reduced after drug pretreatment. Thus *O sanctum* appears to prevent alarm reaction which induced rise in 5-HT. The effect of stress on brain 5-HT level may have its implications in the genesis of stress related disorders, because it has been found that in Schizophrenia, a multifactorial disease, environmental stress being the most important factor, a defect in 5-HT metabolism occurs (Wooley *et al.*, 1954). Brain monoamine oxidase decreases during stress and *O sanctum* has been found to prevent this stress induced change. The stress induced central neurohumoral changes are normalised by *O. sanctum* by modulating these changes of stress.

'Shilajit' is a pale brown exudation of variable consistency, from steep rocks (1000-5000 m) of different formations, found throughout the Himalayas from Arunachal Pradesh in the east to Kashmir in the west. It has also been found in the mountains of other countries like Afghanistan, China, Mongolia and the U.S.S.R. Shilajit is widely used in oriental medicine to arrest ageing and to accelerate the process of rejuvenation—the two major attributes of an Ayurvedic Rasaayan. Until recently there has been considerable controversy regarding chemical constituents of Shilajit and their source material. The first major advance in our understanding of the chemistry of Shilajit was the observation that it contained a large number and variety of organic compounds that can be broadly grouped into humic (80- 85% of total organic mass) and non-humic (20-15%) substances (Ghosal *et al.*, 1991). The non-humic compounds of Shilajit are low molecular weight organic compounds several of which have been isolated and characterized. Soil humus is produced by interaction of plant constituents (from plant exudates and debris) with rhizophere microorganisms. Such interactions with a number of latex and resin bearing plants e.g. *Euphorbia royleana* (Euphorbiaceae), *Trifolium repens* (Leguminosae), *Rhus succedanea* and *Pistasia integerrima* (Anacardiaceae) occurring at different altitudes in Himalayas have been shown to contribute to the formation of some important class of low molecular weight compounds e.g. oxygenated dibenzo (α-pyrones and tirucallane type triterpene acids (Ghosal *et al.*, 1990).

People in the Himalayas use Shilajit to combat cold stress and as a tonic (Tiwari *et al*, 1973). Shilajit apart from being used in diverse clinical conditions in Ayurveda, has also been used to arrest ageing and induce rejuvenation and to improve memory, major attributes of Ayurvedic Rasaayana and Medha Rasaayana (Sharma, 1978).

Shilajit possesses cell stabilising action and this mast cell protecting action by Shilajit is against a number of challengers and prevents the antigen induced degranulation of sensitized mast cells (Ghosh *et al.*, 1989). Shilajit significantly decreased the restraint stress ulcer index, in pylorus ligated albino rats, compared to the control and the Aspirin treated group (Ghosal *et al.*, 1988). Anti-inflammatory and analgesic activity has been recorded (Acharya *et al.*, 1988). The observed neurochemical effects induced by Shilajit, indicated a decrease in rat brain 5-HT turnover, associated with an increase in dopaminergic activity, helps to explain the observed nootropic and anxiolytic effects of the drug (Jaiswal & Bhattacharya, 1992).

In ancient Ayurvedic texts 'Medhya Rasayana' materials are supposed to counteract the effect of mental stress by tranquilizing the users and by improving their memory span and intelligence. Charak, under the heading 'Medhya Rasayana' has enumerated Manduk-Parni botanically referred to as *Centella asiatica* syn. *Hydrocotyl asiatica* Linn (family Umbelliferae) as one of such drugs. It is a prostrate, perennial, faintly aromatic herb found as a weed in marshy places throughout India upto 600 m elevation. In folk medicine the herb is being exten-

sively used in various parts of India for various ailments like headache, bodyache, insanity, asthma, as antileprotic and in wound healing (Chopra *et al.*, 1956).

C. asiatica has been used for similar diseases in the traditional systems of medicine in some other countries (Singh *et al.*, 1984). The leaves are recorded as official drug in the Dutch, French, Mexican, Spanish and Venezulean Pharmacopoeias (Quisumbing 1951) and also included in Extra Pharmacopoeia (Martindale, 1982) and Indian Pharmaceutical Codex (Mukherji, 1953).

Alcoholic extract of the whole plant exhibits CNS depressant effect in albino rats. It produced hypothermia and varying degrees of sedation proportional to the concentration of the drug. Besides exhibiting a barbiturate hypnosis potentiation effect, the drug also showed anticonvulsive activity. The sedative action of the drug was attributed to the glycosidal saponin (Ramaswamy *et al.*, 1970), the mode of action being mainly on the cholinergic mechanism in the central nervous system. In addition to sedative properties, the drug is found to possess an antidepressant action which appeared to be mediated through D_2 receptor and a cholinomietic action which is blocked by atropine and chlorpromazine (Sakina & Dandiya, 1990). The effect of the whole dried herb, on the general mental ability of mentally retarded children was determined by Apparao *et al.*, (1973). The double blind study showed that within the treatment period of three months, the drug produced significant intellectual improvement and behavioural changes. The IQ of children increased 6% as compared to a 3% increase in the placebo group The drug increased power of concentration and attention in children The shy and withdrawn who were restless and fidgety, became expressive, communicative and cooperative The behavioural improvement compared favourably with that noticed with glutamic acid, vitamin or Encephabol (Ramachandran, 1980) The drug could therefore be used for treatment of mental retardation and as a brain tonic (Kakkar, 1990) The aqueous extract of *C. asiatica* (25 mg/kg/i.p.) decreases spontaneous motor activity and delays pentylenetetrazole induced convulsions in mice and the activity has been observed to be comparable to diazepam The extract potentiated pentobarbitone induced sleep time but did not affect immobility time in swimming test — the extract thus has anti-anxiety effect but does not affect the behavioural despair (Diwan *et al.*, 1991).

Bacopa monniera Linn. (syn. *Herpestis monniera* H.B. & K.) (family Scrophulariaceae) popularly known as Brahmi is an annual creeping plant found throughout India in damp and marshy areas. The plant is a nervine tonic used in asthma, epilepsy and insanity. From the ancient Hindu texts it is found that Brahmi has been used as a specific medicine for the development and improvement of memory. The defatted alcoholic extract of Brahmi improves the learning process and corrects to some extent the abnormal behaviour in epileptic patients. Improvement of alpha index in patients following prolonged administration of Brahmi and improvement of learning process has been further substantiated by experiments on treatment with Brahmi for 24 days exhibiting maximum improvement in the

maze-learning of albino rats. Brahmi has been shown to cause prolonged elevated level of cerebral glutamic acid and a transient increase in GABA level. It is thus assumed that an endogenous increase in brain glutamate may be helpful in the process of learning, but the exact mechanism of action is yet to be known (Shukla *et al.*, 1987).

A comparative study of the effect of alcoholic extract of Brahmi and chlorpromazine on the process of motor learning in rats revealed improvement in their performance (Prakash and Sirsi, 1962). The tranquillizing property of the extract was found to be weaker than that of chlorpromazine (Ganguly and Malhotra, 1969). The total extract and the alkaloidal fraction inhibited and respiration of rat brain tissue to a varying degree. LSD-25 and 5-HT did not influence the inhibitory effect of the alkaloidal fraction (Dhalla *et al.*, 1961).

Hersaponin, a glycoside isolated from the plant showed a sedative effect in mice. It potentiated hypnosis produced by hexabarbital, pentobarbital and ethanol in mice and delayed the rate of disappearance of blood pentobarbital sodium in dogs (Malhotra *et al.*, 1960; Malhotra *et al.*, 1962). Hersaponin was found to deplete the rat brain of its noradrenaline and 5-HT content, the action being similar to that of reserpine (Malhotra *et al.*, 1960 & 1961).

Convolvulus pluricaulis Chois. (family Convolvulaceae) popularly known as 'Sankhpushpi' is one of the Medhya Rasayana drugs described in Ayurveda. The drug is advocated as a brain tonic and a remedy for treatment of mental disorder. A report on the clinical trials indicated anti-anxiety effect of the drug. The total extract has been reported to possess barbiturate hypnosis potentiation effect.

Nardostachys jatamansi DC. (family Valerianaceae) is an erect perennial herb with long stout woody rootstock found in alpine Himalayas, 3500 to 5000 m extending eastwards from Himachal to Sikkim and Bhutan. The root stock contains essential oil and jatamansone as the major ketonic sesquiterpene present in the oil. In Ayurveda, besides other uses, the drug has been mentioned to increase intellect/memory, removes evil spirits and oedema and gives strength. Traditionally the drug has been used in epilepsy, hysteria and convulsions. The oil potentiated phenobarbital narcosis in rats, reduced rat brain serotonin content and decreased the conditioned avoidance performance in cats (Hamied *et al.*, 1962). Jatamansone was shown to exert tranquillizing activity in mice and monkeys. Further studies on the effect of jatamansone on the biosynthesis and metabolism of serotonin in rabbit brain revealed an impairment of biosynthesis of serotonin in the brain tissue, thus leading to a reduction in the brain levels of 5-hydroxytryptamine. The degradation of serotonin was unaffected. The mode of action of jatamansone was thus at variance with that of reserpine which has a direct action on the cell to liberate serotonin (Arora, 1962).

In a double blind clinical trial with jatamansone and placebo in 28 hyperkinetic children (below the age of 14 years) for a period of 11 months in comparison with

two standard drugs viz. D-amphetamine and chlorpromazine, both jatamansone and D-amphetamine showed significant improvement in the behaviour of children Jatamansone showed less improvement in restlessness and aggressiveness, as compared to D-amphetamine. Hyperactivity, however, responded equally favourably to jatamansone and D-amphetamine. Children with marked mental retardation showed lesser response to all the three drugs. The results with placebo were very inconsistent. The patients showed improvement in behaviour (Gupta and Virmani 1968). With this drug mental symptoms of the schizophrenic patients were found reduced (Mahal *et al.*, 1976).

Valerian consisting of roots and rhizomes of *Valeriana officinalis* (European valerian) and *V. wallichii* (India valerian) is used as sedative and tranquillizer in the form of powder, tincture and extracts for the treatment of hysteria, hypochondriasis, nervous unrest and similar emotional states. Valepotriates, a group of iridoid monoterpene derivatives were first isolated from *V. wallichii* by Thies & Funke in 1966 and this was followed by investigations on *V. officinalis* and *V. wallichii* in a number of countries particularly Germany, Bulgaria, Netherlands, Denmark, Poland and Russia. European pharmaceutical industry lists over a hundred hypnotic sedative preparations containing valerian extracts and valepotriates as well as number of cardiac formulations containing valerian Pharmacopoeias of France, Germany, Switzerland, England and U.S.A. have *V. officinalis* as official drug, whereas Indian Pharmacopoeia had prior to 1966 both the species official but after 1966 only *V. wallichii* was retained. Valepotriates possesses definite sedative and tranquillizing activity in mice, cats and humans Such effects are not synergistic with those of alcohol and barbiturates and this property is particularly significant in view of the problems often encountered with other sedatives and tranquillizers. Cognitive functions have been studied in twenty previously untreated epileptics on sodium valproate monotherapy. There was observed progressive improvement in attention, immediate recall, intelligence and visuo spatial functions, after drug therapy but the orientation, recent and remote memory remained unaltered. None of the patients showed impairment in cognition and the improvement in cognition was apparently due to adequate seizure control (Jha *et al.*, 1992). Valepotriates are triesters of polyhydroxy alcohol with isovaleric, acetic and isocarproic acids. However, the alcohol as such does not exist in nature. The word Valepotriate is formed by combination of three radicals: Val, for those substances which have been discovered from Valerian, epo-, as they have an epoxy function and triate-, to indicate that they are triesters. Valepotriates are unstable and undergo rapid decomposition at temperature 40°C and are destroyed by mineral acids (*p*H 3) and alkalies (*p*H 11). Keeping in view their highly sensitive nature, their processing has to be done very carefully (Gupta *et al.*, 1986). Different types and races of the plant are attributed to natural diploid, tetraploid and octaploid types of the plant. In France octaploid species *V. sambucifolia* is cultivated and in Germany tetraploid species. *V. collina* is cultivated. In India no cultivation is done but the wild species

V. wallichii contains 2% of Valepotriates as compared to European species *V. officinalis* which contains 0.5% Valepotriates. The root of peruvian species *V. thalictroidis* has been reported to contain 14.6% but the roots are very thin (Becker *et al.*, 1983).

The rhizomes of *Acorus calamus* Linn. (family Araceae) popularly known as 'Bach' collected during autumn, have been used for the treatment of epilepsy and other mental disorders like hysteria, insomnia etc., either as a single drug or as a component of certain compound drug preparations in the Ayurvedic system of medicine. The plant grows in East Asia, Europe and North America. The East Asia race is tetraploid containing 6% volatile oil with 20% β-asarone content. The European race is triploid containing 4% volatile oil with 10% β-asarone content. The North America race is diploid containing 2.2% volatile oil which is practically free from asarone content. Because of carcinogenicity of arasone, the F.D.A. has prohibited the use of calamus in the U.S.A. However, it is suggested that the diploid American variety is the only alternative for the use of the drug. Since *Acorus calamus* oil has tranquillizing and sedative properties comparable to chlorpromazine and reserpine, it is used for the treatment of epilepsy and depression in India. In Ayurveda, the drug is specifically mentioned for children as an antiepileptic psychoactive drug and is reported to cure insanity, promote intellect and intelligence. The essential oil and the crude alcoholic and aqueous extracts obtained from the roots exhibited depressant action in dog and the essential oil was found to be more active than the other two extracts (Bose *et al.*, 1960). The essential oil when given intraperitoneally afforded protection against development of hind limb tonic extension in electrically induced convulsions but it was ineffective in metrazole induced convulsions (Madan *et al.*, 1960). The essential oil also showed relaxant effect as well as an antispasmodic action against various spasmogens in various organs of different species (Das *et al.*, 1962).

Further studies on the essential oil of *A. calamus* have yielded two active principles namely, α-asarone and β-asarone which prolonged the hypnosis induced by pentobarbital, hexobarbital and ethanol in mice. Asarone prevented depletion of adrenal ascorbic acid in rats subjected to cold stress and did not cause any change in the noradrenaline content of whole brain of rats (Menon & Dandiya, 1967). Asarone failed to cause any change in serotonin level of the rat brain (Dhalla and Bhattacharya, 1968). Aqueous and alcoholic extract of *Acorus calamus* has been proposed for treatment of 'petitmal' epilepsy (Martis *et al.*, 1991).

Hypericum perforatum Linn. (family Clusiaceae; Guttiferae) is a perennial herb growing wild in hedge rows, rough grassland, open woodland and is most common St. John's wort in Britain. Experimental animal studies of the psychotric activity of hypericum extract have been done. Extracts of *Hypericum perforatum* (Psychotonin^{R})with known concentrations of hypericin were tested in several models generally accepted as screening methods in experimental

animal studies for the recognition of psychotropic, and in particular of antidepressant activity. Hypericum extract enhanced the exploratory activity of mice in a foreign environment, significantly prolonged the narcotic sleeping time dose dependently, and within a narrow dose range exhibited reserpine antagonism. Similar to most other antidepressants, hypericum extract enhanced significantly the activity of mice in the water wheel test and after prolonged daily administration decreased aggressiveness in socially isolated male mice. This experimental data in addition to the already proven clinical efficacy justify the use of standardized Hypericum extract in the treatment of mild to moderate depression (Okpanyi and Weischer, 1987). The plant extract had an LD_{50} of 1000 mg/kg i.p. in rats (Sharma *et al.*, 1978).

Cannabis sativa Linn. (family Moraceae) appears to be a source of an antiepileptic drug. There are two chemotypes in this plant. The one which exerts hallucination is rich in Tetrahydrocannabinol (THC) and is thus referred to as THC type The other is CBD type which is devoid of hallucination and rich in cannabidiol. Although structure of cannabidiol was determined in 1963, only sporadic biological work on it has been reported as it is not psychotropic. Cannabidiol has been reported to be potent antiepileptic although at relatively higher doses Clinical trials in patients with grand mal epileptic attacks which were refractive to the generally employed antiepileptic drugs were given 200 mg daily dose of cannabidiol over several months. In most patients the convulsive attacks either disappeared or became much less frequent (Mechoulam and Lander, 1980). This could be a possible source of drug for epilepsy. Some studies have shown that *Picrorhiza kurroa* is a potent immunostimulant, stimulating both specific and nonspecific immune mechanisms and *Tinospora cordifolia* improved the phagocytic activity without affecting the humoralor cell mediated immunity (Atal *et al.*, 1986; Simons *et al.*, 1989).

One of the oldest Chinese therapeutic agents used is a leaf extract of the 'fossil tree', *Ginkgo biloba*. This tree has long been part of the traditional Chinese pharmacopoeia, being first cited as medicinal agent some 5000 years ago (Braquet, 1988). Although the traditional medicinal properties of *G. biloba* have been known for many centuries, it is only recently that they have been explained in terms of modern pharmacology (Braquet 1989). *Ginkgo biloba* extract (G.B.E) is defined and patented extract of *G. biloba* leaves. Its trade name is Rokan^R in Germany and Tanakan^R in France, marketed by Ipsen-Beaurour, Paris, France. The concentrated extract essentially contains terpenes, proanthocyanidins and flavoglycosides. Ginkgolide, the major active principle was first isolated by Furukawa in 1932 and the structure resolved in 1967 by the pioneering work of Nakanishi and colleagues. These unique 20-carbon cage molecules which incorporate a tert-butyl group and six 5-membered rings, are specific and potent antagonists of platelet activating factor (PAF), an anti-inflammatory autocoid that is produced by, and acts on, a wide variety of cell types (Braquet,

1987; Braquet and Hosford, 1991). Both animal and human pharmacological studies have revealed interesting actions of *Ginkgo biloba* extract in relation to the restoration of regional cerebral blood flow after microscopic or spontaneous appearing embolism. In addition, energy metabolism is improved in case of ischaemia, with a consequent improvement of cerebral electrogenesis. G.B E. has also shown antioedema action in the brain after microembolism. In humans, G.B.E. reduces the mean time of brain perfusion and thus increases the oxygen and glucose consumption of the brain. These findings show that G.B.E. medication has a positive effect in geriartic subjects with deterioration of mental performance and vigilance and this effect is reflected at the behavioural level. These results have been revealed by a study of the long-term action of G.B.E. on vigilance and mental performance as determined by means of quantitative pharmaco-EEG and Psychometric measurements (Gebner *et al.*, 1985).

Ginseng represents the roots of perennial plant *Panax ginseng* (Chinese ginseng). *P. quinquefolius* (American ginseng), *Eleutherococcus senticosus* (Siberean ginseng), Korean ginseng and Japanese ginseng. These have been used widely in traditional oriental medicine due to its stimulating and adaptogenic activities. Numerous phytochemical and pharmacological studies reveal that the major active ingredients of ginseng are ginsenosides and panaxosides. Neuropharmacological effects of ginseng reported by Brekhman and Dardymov (1969) were further substantiated by the evidence which indicates that ginsenosides affect behaviour and brain function and these phytoconstituents have been shown to specifically inhibit the uptake of various neurotransmitters into brain synaptosomes in a dose dependent manner (Tsang *et al.*, 1985). Total ginsenosides, at concentration ranging from 0 001 to 50 μg/ml, reduced the high K^+-evoked release ^3H Noradrenaline in calcium-containing but not in a calcium-free media, and this inhibition was concentration dependent It, however, failed to affect the release of ^3H Noradrenaline significantly from undepolarised samples in Krebs-Ringer-bicarbonate media and such results support that the ginsenosides may affect behaviour and brain function (Tsang *et al.*, 1986).

Some of the Rasaayana and Medhya drugs like *Withania somnifera*, *Ocimum sanctum*, *Tinospora cordifolia*, Shilajit, *Centella asiatica*, *Bacopa monniera*, *Convolvulus pluricaulis*, *Nardostachys jatamansi* and *Acorus calamus* mentioned here have provided preliminary leads validating the claims made in Ayurveda. It is therefore essential to select such plants one by one and thoroughly investigate them both clinically and experimentally so that fruitful outcome is achieved in building the defence system of the body and rejuvenation therapy.

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Studies towards the Development of a New Anti-inflammatory Drug from *Boswellia serrata* Gum Resin

S.C. Taneja & K.L. Dhar

Regional Research Laboratory, Jammu - 180 001

DISCOVERY of new drugs from natural sources such as plants and marine organisms is gaining increasing importance in developed as well as developing world. It is an accepted fact that drugs presently in clinical use as anti-inflammatory are far from satisfactory, though they can effect and provide partial relief from unpleasant symptoms such as pain, heat or swelling, they have little or no effect in reversing the degenerative process of most rheumatic diseases. These drugs are mostly associated with undesirable side effects which in extreme circumstances can be life threatening. Therefore, search for newer and more effective anti-inflammatory drugs is an important and major activities of many R&D organisations.

Inflammation

To a layman inflammation often means heat of a part of body with pain, redness and swelling. Inflammation if acute is not a disease but a defensive response to injury. However, if acute inflammation persists, it evolves to chronic inflammation which is often systemic and biochemically different from acute inflammation. Inflammation has a direct relationship with rheumatic and arthritic diseases. The rheumatic diseases may be defined as those conditions characterised by pain or stiffness of some parts of the musculoskeletal system. The term arthritis is used to designate the group of conditions in which the articular structure are the primary ... many of the rheumatic diseases are

characterised not only by signs and symptoms of articular involvement but by diffuse manifestation of connective tissue disease. As most of these diseases are chronic in nature with variable cause manifested by spontaneous remission and exacerbations, the evaluation of a therapeutic agent is very difficult. This difficulty in critical evalution accounts for short-lived success of many therapeutic agents.

Medicinal plants especially those used in the ancient system of medicine such as Ayurvedic, Yunani, Chinese etc., for the treatment of inflammatory diseases are useful source of new lead molecules. Many such leads have been followed by research workers and some of these works have been reviewed[1,2]. Similarly plants with some folkloric importance may also prove to be equally good starting materials for new lead structures. Most drugs prescribed by the practitioners of traditional system of medicine are in the form of crude extracts wherein the identity of the constituents are not known. Therefore, the need of identification of the active principles, study of their pharmacology, toxicity and standardisation of methods of extraction etc., is an essential requirement for the consistency in biological activity as well as the active constituents

Boswellia serrata Roxb. (family Burseraceae) is a tree of economical value [3,4]. It is also popularly known as salai guggal, white guggal, Indian olibanum or dhup. The research and development work carried out on the gum resin of the tree towards the development of new anti-inflammatory and antiarthritic drug is a small step towards this direction. In the following lines a brief history of some of the work done in chemistry and pharmacology has been reviewed.

Boswellia serrata is a deciduous middle-sized tree which is mostly concentrated in tropical parts of Asia and Africa. In India it occurs in dry hilly forests of Rajasthan, Madhya Pradesh, Gujarat, Bihar, Assam, Orissa as well as central peninsular regions of Andhra Pradesh, Assam etc. The gum is tapped from the incisions made on the trunk of the tree, which is then stored in specially made bamboo baskets and converted into dfferent grades of material according to flavour, colour, shape and size.

The fresh gum obtained from the tree is hot dry with a pleasant flavour and slightly bitter in taste. It is the 'frankincense' of ancient Egyptians, Greeks and Romans who used it as prized incense, fumigant as well as multipurpose aromatic. It is generally used in making incense powders and sticks.

The oleo-gum resin of *Boswellia serrata* is used in various Unani and Ayurvedic preparations. It is reported to be useful in the treatment of bronchitis, asthma, cough, bad-throat and various intenstinal problems. It is diaphoretic and astringent and prescribed in various syphilitic and pulmonary diseases. It acts both as internal and external stimulant, expectorant, diuretic and stomachic. Gum is also prescribed in cases of jaundice, diarrhoea, dysentry, dyspepsia and haemorrhoides. It is also recommended in weak and unhealthy kinds of ulceration[5].

Chemistry of *Boswellia serrata* Gum Resin

The chemistry and pharmacology of the gum resin has been the subject of a review in the past[6] The ole-gum-resin is a complex mixture of terpenoids and sugars. In broad terms the chemical constituents may be divided into three main groups.

1 Volatile oil or lower terpenoids

2. Higher terpenoids

3 Carbohydrates.

1. Volatile Oil or Lower Terpenoids

Steam distillation of fresh gum resin yields upto 10-16% of essential oil with characteristic odour. Physico-chemical characteristics of the volatile oil depends upon the source material. The monoterpenes identified in the oil include α-pinene, β-pinene, α-thujene, δ-limonene, ρ-cymene, cadinene, geraniol, elemol, terpeneol, methyl chavicol and phellandrene[7-12].

2. Higher Terpenoids

Higher terpenoids constitute the major fraction (25-35%) of the oleo-gum-resin. A good amount of work has been carried out on the chemistry of higher terpenoids since the first isolation of boswellic acid in 1898 by Tschirch *et al.*[13] Since then number of chemists have worked on the structure elucidation of β-boswellic acid, some eminent names include Ruzicka, Ferdinado, Simpson, Bilham *et al.*[14-21] Graham Allen[22,23] provided the chemical evidence for disclosing the stereo-identity of hydroxyl and carboxyl functions and configuration at C-5, C-8, C-10, C-13 and C-17. He finally established that configuration of hydroxyl and carboxyl groups in β-boswellic acid is axial. Structure of methyl ester of acetyl-β-boswellic acid has also been confirmed by single crystal X-ray studies recently[24]. β-boswellic acid has some characteristic features in its structure. It belongs to ursane group of triterpenoids with C-3 hydroxyl function oriented axially which is not very common in this group. Moreover there is a carboxylic function at C-4 which is also oriented axially making ring A little strained. The bond angle between C-5, C-4 and C-24 is 115.9°. The axially oriented hydroxyl and carboxyl groups make the molecule more susceptible to certain chemical transformations e.g. oxidation to 3-keto derivative invariably leads to decarboxylation.

Besides, α-boswellic acid several other triterpenoids have also been isolated from the gum resin, these compounds include α-amyrins, 11-keto-α-boswellic acid, acetyl-11-keto-α-boswellic acid, 3′-hydroxy urs-9, 11-dien-24-oic acid, 3′-acetoxy urs-9, 11-dien-24-oic acid (Fig. 1). Tetracyclic triterpenoic acids have, also been reported by Pardhy *et al.*[25-27], these compounds are 3′-hydroxy-tirucall-8, 24-dien-21-oic acid 3′-hydroxy-tirucall-8, 24-dien-21-oic acid, 3-keto-tirucall-8, 24-dien-21-oic acid, and 3′-acetoxy tirucall-8, 24-dien-21-oic acid (Fig. 2). Besides, these authors have also reported the isolation of a new diterpene alcohol serratol (Fig. 3).

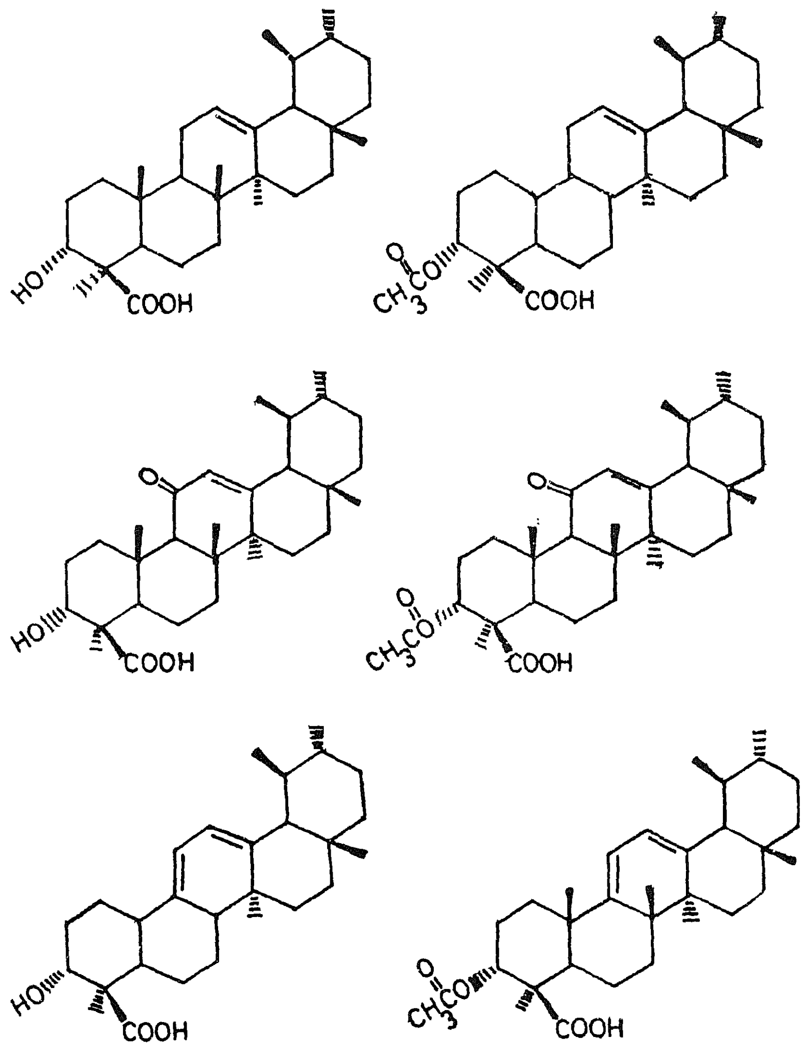

Fig.1. BOSWELLIC ACIDS

More recently Mahajan *et al.*,[28] have reported the isolation and structure elucidation of two new triterpenoids from acidic and neutral fractions of the gum extract, the structure of these compounds are 2′-3′-dihydroxy urs-12-ene-24-oic acid and

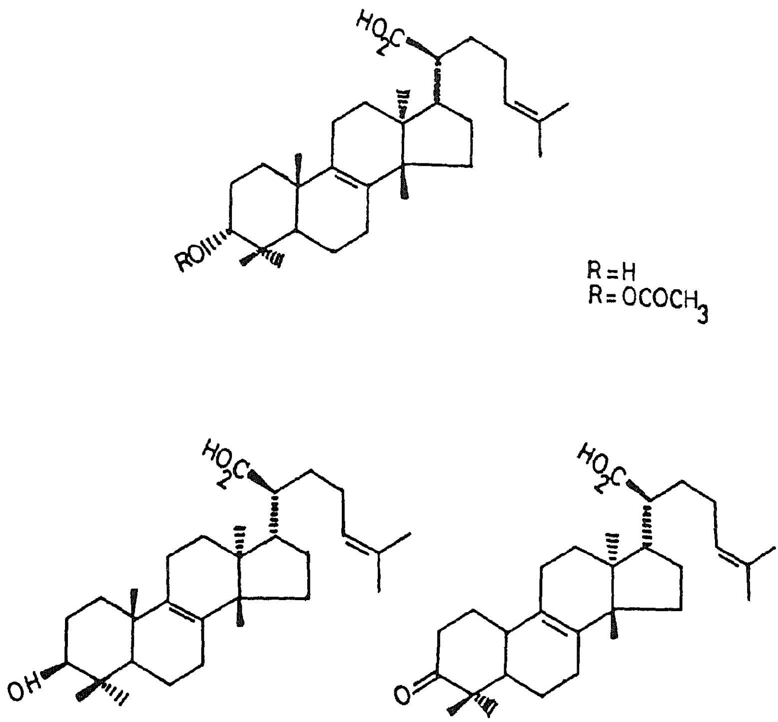

Fig.2 TETRACYCLIC TRITERPENOIDS

urs-12-ene-3′-24-diol[25]. Presence of another diol viz. urs-12-ene-3α-24-diol has also been confirmed in the neutral fraction (Fig. 4)[29].

3. Carbohydrates

Carbohydrates or sugars also constitute the major fraction (45-60%) of the gum resin. Preliminary examination indicated the presence of disachharides along with oligo- and polysachharides. Although not much work has been carried out to determine the compositions of sugars present in the gum resin some earlier work reported by Malandkar[30] *et al.*, identified the acid hydrolysed products as arabinose, xylose and galactose.

Methods of Isolation

Extensive pharmacology of different extracts of the gum resin has led to the identification of the active fraction. This fraction constitute mainly the triterpenoic acids which include both pentacyclic and tetracyclic triterpenoic acids. For the

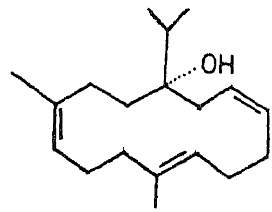

Fig.3. SERRATOL

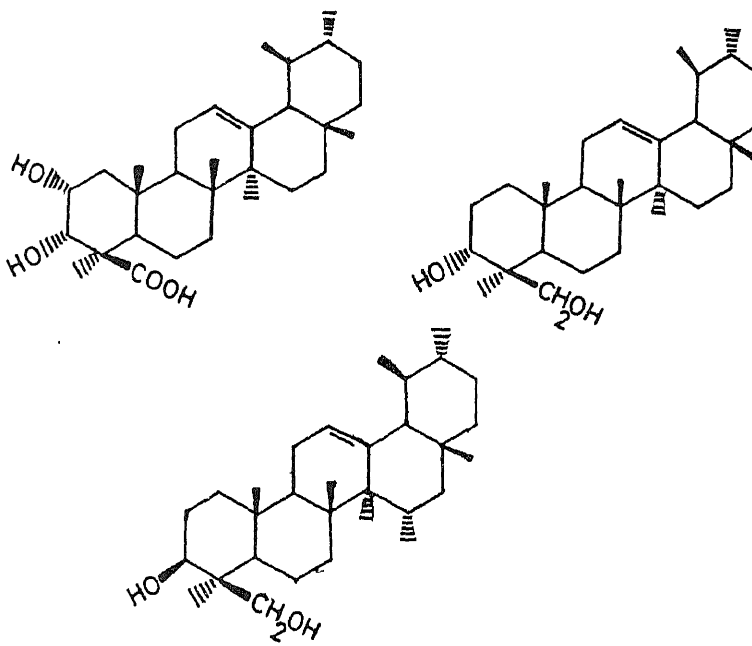

Fig.4. NEW PENTACYCLIC TRITERPENOIDS

extraction and separation of triterpenoic acid fraction (BA) from the gum-resin following methods may be adopted:

Method 1

Gum resin of *B. serrata* which is crushed to small lumps or coarse powder is first extracted with n-hexane or petroleum ether and then with methanol. The methanolic extract is separated into neutral and acidic fractions by treatment with alkali. Further separation of individual acids may be achieved by column chromatography over silica gel (Flow sheet 1).

Method 2

Triterpenoic acids in the form of acetates may be isolated by the modified method of Winterstein[31] *et al.* The crushed lumps or powdered gum exudate are extracted with solvent of medium polarity such as diethyl ether, acetone or ethyl acetate etc., and the total acids are precipitated as their barium salts. The barium salt on treatment with acetic-anhydride form a mixture of triterpenoic acid acetates. Repeated crystallisation from the mixture results in the separations of acetyl-β-boswellic acid and acetyl-11-keto-β-boswellic acid in pure form. These mixtures may also be separated by column chromatography over silica gel. Pure β-boswellic acid and 11-keto β-boswellic acid are obtained by acid or alkaline hydrolysis of the corresponding acetates (Flow sheet - 2).

Pharmacology

It has already been mentioned that the oleo-gum- resin of *B. serrata* has long been in use in traditional system of medicine. It also has a folklore reputation in treatment of arthritic diseases. Extracts of the gum resin have shown potent anti-inflammatory and antiarthritic activities in laboratory animals as well as during clinical trials[33,34]. In a detailed investigation Singh *et al.* [35,36] have conclusively established that defatted alcoholic extract of the gum resin (95% v/v) has marked antiinflammatory activity in carageenan induced oedema in rats and mice and dextran oedema in rats. It was also effective in formaldehyde induced arthritis in rats. When dosed orally over a range of 50-200 mg/kg it was found to be as effective as phenylbutazone. These results were also repeated in normal and adrenalactomized rats. However it was not found active in cotton wool pellet granuloma test.

Detailed pharmacology of the acid fraction (BA) separated from the total extract was also conducted on similar lines[37]. BA also demonstrated a dose related antiinflammatory activity in various test models. In a dose range of 25-200 mg/kg orally the acid fraction displayed 25.71 to 47.54% inhibitory action in carageenan, histamine and dextran induced oedema in rats and mice. The former test being widely employed for its sensitivity and reliability for the evaluation of drugs of proven value. Anti-inflammatory action remained unaltered in adrenalactomised rats indicating its action independent of activation through pituary- adernal axis. In acetic acid induced vascular permeability test BA (50-200 mg/kg PO) produced 41.94 to 58.53% inhibitory effect with P.value < 0.001. In chronic test of developing adjuvant arthritis, the fraction demonstrated antiarthritic activity in the range of 32.23 to 54.54% with significant inhibition of secondary lesions, in result it checked the loss of body weight, nodule formation on tail and haemorrhagic patches on ears.

BA was found equally effective in established adjuvant arthritis indicating its possible usefulness as an antiarthritic agent. This effect was more marked in chemically induced formaldehyde arthritis in rats. The BA at dosage range of 50 - 200 mg/kg produced 37.54 to 62.85% inhibitory effect. In sodium urate gouty

Oleo-gum-resin
Hexane pet-ether

Soluble

Insoluble
Methanol

Soluble
Alkali

Insoluble

Neutral Fraction

Acidic Fraction
Column
chromatography

Flow Sheet - 1

Ole-gum-Resin
Solvent ether/Acetone/ethyl acetate

Insoluble

Soluble
Barium hydroxide

Soluble

Precipitate
Acetic anhydride
Acetate mixture

Column
Chromatograph

Crystallisation

Acetyl-β-boswellic acid
Acetyl-11-keto-β boswell acid
and acetates of other acids
Hydrolysis
β-boswellic acid,11-keto-
β-boswellic acid, other acids

Acetyl-β-boswellic acid
Hydrolysis
β-boswellic acid

Flow Sheet - 2

Table 1 — Physical data of higher terpenoids from *Boswellia serrata* gum resin

S. No.	Compound	M.P.(°C)	[α]D
1.	Amyrin	186	+91
2.	α-amyrin	197	+79
3.	α-boswellic acid	232	+107
4.	Acetyl-α-boswellic acid	271-73	+63
5.	11-keto-α-boswellic acid	270	+128
6.	Acetyl-11-keto-α-boswellic acid	238-46	+87
7.	3α-acetoxy urs-9,11-dien-24-oic acid	175*	+338*
8.	3-keto-tirucall-8,29-dien-21-oic acid	212	+28*
9.	3′-hydroxy-tirucall-8,24-dien-21-oic acid	149-50	-11*
10	3′-acetoxy-tirucall-8,24-dien-21-oic acid	220	-44*
11.	3α-hydroxy-tirucall-8,24-dien-21-oic acid	188	-2*
12.	2′-3′-dihydroxy urs-12-ene-24-oic acid	174	-
13.	Urs-12-ene-3′-24 diol	184	+69
14.	Urs-12-ene-3′,24 diol	180	-
15.	Serratol	-	-93

* Methyl ester[32]

arthritis in dogs, BA at a dose level of 100-300 mg/kg decreased the total leucocytes in the aspirated synovial fluid from 20.60 to 57.41% and it checked the development of typical three legged walk in comparison to the control dogs which evinced pain when made to walk.

In bowine serum albumin (BSA) induced arthritis in rabbits, BA at doses 25, 50 and 100 mg/kg orally, significantly reduced the population of leucocytes in BA injected knees. It was also found to be equally effective upon injecting it locally into the knee at doses 5,10 and 20 mg/kg.

It was also observed that acid fraction significantly inhibited the migration of leucocytes into pleural cavity, pleurisy induced by carageenan and dextran. It displayed no action on haemolysing activity, local irritations or cytotoxic effect as evidenced by dye exclusion test model. The fraction inhibited the arthritis elevated level of connective tissue metabolites in urinary excretion hydroxyproline (free, total, non-dialysable and dialysable) hexosamine and urinic acid.

Safety evaluation and preclinical toxicology of BA was also conducted during detailed pharmacological investigations. Daily oral administration of BA in three dose levels low, medium and very high doses in rats was conducted and various haematological and biochemical parameters were determined in control and drug treated rats. Histopathology of various vital organs obtained from these rats revealed no change in architecture of different tissue cells, indicating the sefety of BA even in very high doses on prolonged treatment.

Mechanism of Action

(a) Anti-inflammatory Activity on New Papaya Latex Model

Gupta *et al.*[39,39] have developed a new model employing latex of papaya as an inflammagen for testing the anti-inflammatory activity. The inflammation caused by hydrolytic enzymes present in the latex mimic the clinically encountered inflammatory conditions in which lysosomal enzymes have been reported to play an important role. The new model has displayed its sensitivity and reliability in a wide range of anti-inflammatory drugs. The BA from *B. serrata* when tested on this new model, showed significant activity of mean 35% inhibition of inflammation. Since the new model is reported to be sensitive to slowly acting, remission inducing drugs, its effectiveness on BA throws some light on its mechanism of action which seems to be unlike aspirin and steroidal drugs.

(b) Anti-complementary Activity

Since complement is one of the important mediators of the inflammatory reponse it was assumed that inhibition of complementary activity would be expected to inhibit inflammatory models in which complement activation is involved. BA from the gum resin was found to possess anti-complementary activity[4]. It inhibited *in vivo* immunohaemolysis of antibody coated sheep erythrocytes by pooled gunea pig serum. The reduced immunohaemolysis was found to be due to inhibition of C-3 convertase of classical complement pathway. The threshold concentration for inhibition of C-3 convertase was found to be 100 ug. However higher concentration of BA showed constant inhibitory effects on immunohaemolysis. It also exhibited weak inhibitory effect on individual components of the complement system. *In vivo* administration of BA also showed inhibitory effect on gunea pig serum.

(c) Leukotriene Inhibition

It has also been recognised by Ammon *et al.*[41], that ethanolic extract of the gum resin inhibits the formation of Leukotriene B_4 in rat peritoneal neurophils. Leukotriene such as LTB_4 is recognised as one of the important mediators of inflammatory reactions. Leukotrienes are synthesised by stimulated phygocyte cells, particularly the neurophils and the production of chemostatic factors by these cells attracts more phygocytes to sites of inflammation. Most other non-steroidal antiinflammatory drugs act through the inhibition of prostaglandins (PGE's) produced

by stimulated phygocytes. BA, therefore, is different from other known non-steroidal anti-inflammatory drugs in its mode of action and relatively free from side effects.

Drug of Future

A good amount of money is being pumped in various research and development efforts world over in search of better and safer anti-inflammatory drugs. Hundreds of plant and marine species have been screened in the past and a wide range of molecules have been identified possessing anti-inflammatory and antiarthritic properties. However the war against such diseases is yet to be won.

Detailed pharmacology, biochemical studies, preclinical safety evaluation and toxicological studies have unambiguously established the potential and efficacy of BA as a candidate for a novel anti-inflammatory drug. Most of the non-steroidal anti-inflammatory drugs currently in use are associated with side effects such as ulceration and gastric irritations. These drugs act through prostaglandin pathway which are important mediators of inflammatory reactions. BA on the other hand has a different mode of action as it inhibits the formation of lukotrienes which are also recognised as the mediators of inflammation. BA has also displayed anti-complementary activity both *in vitro* and *in vivo* experiments. It is comparatively free from side effects and on the other hand pharmacological screening revealed some of its beneficial sides where it caused lowering of cholesterol levels along with anti hyperlipidemic and anti-artherosclerotic actions [38,34].

BA has been found to be quite safe at high dose levels. Phase-I clinical trials on human volunteers have already been successfully conducted without finding any adverse effects. With pharmacological data in its support and its natural product origin, BA from *B. serrta* oleo-gum resin is all set to a future anti-inflammatory drug.

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Annatto — The Natural Colour

C. Srinivasulu*

Regional Research Laboratory, Bhubaneswar - 751 013

Introduction

ANNATTO is one of the oldest food colourants which has also been used for colouring textiles like cotton and silk and in cosmetics. It has been prescribed as a medicine for a large number of maladies. Annatto has served as a colourant for butter and cheese for over hundred years. The interest in annatto and other natural colours has been increasing over the years since many of the coal-tar dyes have been found to be carcinogenic.

Annatto is obtained from the seeds of the tree *Bixa orellana* Linn. It belongs to the family *Bixaceae* which is native to tropical America and the West Indies and is naturalised in India. It is known in India as Latkan, Japhra, Rangamale & Sandri. The pods of the tree on drying burst out throwing away the seeds and the dye is present in the thin resin coating of the seeds. The chief colouring constituent of the dye is a carotenoid type of pigment, bixin[1].

Cultivation

The plant is cultivated in Brazil, Guiana, Mexico and the Antilles. In India it is reported to be cultivated to a small extent[1]. The plants are found in Karnataka, Tamilnadu, Andhra Pradesh, Orissa and Maharashtra.

Annatto can be propagated by seeds or vegetatively through mature wood cuttings. Generally, the seeds are sown in April and it takes about 8 to 10 days to

germinate. The seedlings which are about 20 cm tall are planted immediately after the onset of monsoon. The soil has to be mixed with compost and fertilizer, to avoid deficiency of macro and micro nutrients. About 500 plants can be accommodated in a hectare.

The flowers appear in the last week of August and continue upto the middle of October. There are two types, one with white flowers and green capsules, and the other, with pink flowers and red capsules. After about 30 days the trees bear fruits and after 90 days seeds appear in the capsules. The capsules are ready for harvest by January and it is important to harvest them when a crack is found in the stigma end of the capsule. When the capsule is allowed to burst there is heavy loss of seeds. On an average each plant gives about 1/2 kg of seed/yr in the third year and 1 kg from fourth to tenth year. After harvesting, the seeds with pinnacles are packed in gunny bags and kept closed for some days. Then they are sun-dried, the seeds are separated by beating with sticks which are further dried and packed in gunny bags for transport[3].

Extraction Methods

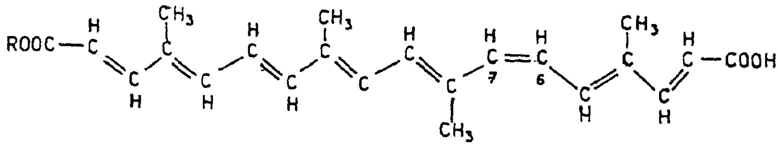
Various methods have been used for extracting the crude colour and bixin. Butter colour is obtained by treating the seeds with a vegetable oil, like castor oil, preferably at 120° [4,5]. The extract is then diluted to specifications.

A concentrate containing 20% bixin was obtained by mechanical agitation of the seeds in an airflow using a spouted bed reactor of capacity ~ 50 kg[6]. According to a report chloroform ethanol (25 : 75) (V/V) gave the highest yield of annatto dye. At a 1:4 seed/solvent ratio (W/V) the extraction yield was 76.48% for 3 extractions. Supercit (supercritical) carbon dioxide has also been used for the extraction of the pigment using pressures of 3,000-7,000 psi and temperature range of 40-55°. Maximum solubility of pure bixin is 0.003 and annatto seed pigment 0.26 mg/g of CO_2. Efficiency of annatto pigment extraction was increased in the presence of oil[7].

A flow sheet has been made for extraction of bixin using ethyl acetate in a soxhlet extractor[8]. Boiling acetone, alcohol and chloroform have also been used. According to one Japanese patent stabilized bixin preparation was obtained by holding bixin in alcoholic solution at *p*H 4.5 for one hour before spray-drying. In another process the seeds or crude annatto colour were stirred with aqueous alkali or aqueous alcohol, filtered and the *p*H of the filterate was adjusted to 5-6 to give bixin.

In one process the colourant was extracted by counter-current method using water during simultaneous mechanical friction on the surface of the seeds. The suspension was evaporated in vacuum, which on subsequent drying and powdering, can be used for colouring various foods like candies.

The commercial cheese colour is an alkaline solution which contains nor-bixin (II) and is obtained by using sodium or potassium hydroxide. Powdered nor-bixin



I, R = CH_3 ; II R = H

has been prepared by spray drying of an 1 to 2% aqueous alkaline solution using a seed-solution ratio of 1:1 - 3 for 10-20 min at ambient temperature (dissolved solid content 10-20° Brix). The spray dryer was operated at an entrance temperature of 150-200° and an exit temperature of 80-100°[10]

Pigment suitable for use in feed and food was obtained by water extraction after treating the seeds with 1 α-glycosidase.

Composition and Chemistry

Bixin (I) is the chief coluring constituent. The proportion of bixin varies from 10-12 % of annatto colour. Special grades containing 15-30% are also known. Orellin is a yellow substance. The seeds contain a small amount of fatty oil. The seed coat contains a wax like substance (3%) which is toxic and paralyses intestinal parasites and the embryo. From the oleoresin of *B. orellana* all -E- geranylgeraniol has been obtained to the extent of 57% or ~ 1% of the weight of dry seeds, which makes it the richest known source of the important c-20 terpene alcohol. Other compounds isolated include farnesyl acetate, geranylgeranyl octadecanoate, geranyl geranyl formate, δ-tocotrienol and an apocarotenoid[11]. β-carotene, cryptoxanthin, lutein. Zeaxanthin A have been tentatively identified by co-t.l.c. It is claimed that annatto seed is a good source of vitamin A because of the presence of β-carotene. The composition of Brazilian annatto seed is given in Table 1[12,13].

The vitamin and mineral content, as well as the fibre fraction, are very similar to those determined in cereals but with a high level of carotenoids. The seeds on steam distillation yields a pungent smelling essential oil (0.08%) which gets polymerised quickly. The composition of the essential oil obtained from Indian seeds is given in Table 2[14].

Isocutellarein was isolated from the hot water extracts of the seeds, which was found to have potent inhibitory activity towards lens aldose reductase. The fatty acid composition was reported to vary depending upon the difference in ecological conditions.

Natural bixin has a Z-configuration between 6 and 7 carbons. It crystallises as violet prisms and melts at 198°. It is extremely sensitive to iodine, in light, to give

Table 1 — Composition of annatto seed

Characteristic	(%)
Protein	12.13-13.12
Ether solubles	5.22-8.84
Pentosans	11.35-14.97
Pectin	0.23-0.55
Ash	5.44-6.92
Total carotenoids	1.21-2.30
β-Carotene	11.30
Crude fibre	14-40

all-E- bixin. They differ in their solubilities and form the basis for oil-soluble and water-soluble annatto colour respectively. Ethyl bixin is used for colouring foods such as margarine, whereas methyl bixin has a poor tinctorial value. Esters of bixin with higher alcohols like amyl, octyl, octadecyl, decyl etc. have been prepared to be used as colouring agents.

Bixin has been converted to a yellow colouring material, by heating with acetic acid in the presence of acetic anhydride, for colouring margarine. m-Xylene, a product of degradation of bixin and methyl bixin, has been detected in commercial samples of annatto colour.

Table 2 — Composition of essential oil

Compound	(%)
Ishwarane	30.7
α-Pinene	9.3
β-Pinene	4.8
α-Elemene	3.3
Valencene	2.7
Amorphene	0.2

Ishwarane and ethyl gallate, leucocyanidin, ellagic acid, 7-bisulphates of apigenin and luteolin and 8-bisulphate of hypolactin (1-OH luteolin) have been isolated from the leaves of the plant whereas tomentosic acid from the root.

Chemotaxonomical studies were made with south Indian Bixaceae and leucoanthocyanidins were found to be present in all the taxa. The distribution patterns of free amino acids phenolic compounds were studied.[15]

Stability

Light was found to be the most destructive agent, followed by benzoyl peroxide. Air was much less effective in promoting loss of colour. Ascorbyl palmitate effectively retarded the destructive effect of light[16].

Detection and Analysis

Bixin was determined by a reproducible spectrophotometric method at 5.50 nm, following chloroform extraction in a tissue homogenizer equipped with a porous glass filter to facilitate the separation of the extract. Using a Zorbax ODS column and solvent systems like water/THF AND THF annatto pigments were separated by high performance liquid chromatography[17]. For the effective separation of annatto carotenoids by column chromatography a 1:1 mixture of silica and diatomaceous earth was used with stepwise elution of solvents with increasing polarity.

In column chromatography bixin is strongly absorbed by alumina, even in the presence of fat. Other carotenoids and artificial colours are not retained by the column.

Detection of annatto on paper is done by using antimony trichloride which gives a blue-green colour. If the dye is present in the lipid phase, it is first saponified and filtered from the unsaponified matter. The filtrate is used for running the chromatogram. Bixin can be detected by the green colour in the carr-price reaction using isopropyl alcohol for elution. With alumina-treated paper petroleum ether-chloroform (4:1) was used as solvent system.

Amyl acetate was used as solvent system for t.l.c. To increase polarity 1% HoAC has been added. For the detection of annatto in citrus juices the sample is extracted with petroleum -ether-alcohol (20:10:13) and the extract was evaporated. The residue was passed on an alumina column and then subjected to t.l.c. using petroleum ether-benzene-acetone-acetic acid (80:20:2:1). In the case of margarine the colour was first extracted with acetone and then subjected to t.l.c. using hexane-acetone-ethanol (20:6:1). Colour was developed with H_2SO_4, HNO_3, $SbCl_3$ in $CHCl_3$, $SbCl_5$ in CCl_4 and $SnCl_2$ in EtOH (100° for 5-10 min).

Utilization

Annatto is used in India generally for colouring butter. About 1 ml of the the commercially available castor oil solution is sufficient for 1 kg of butter. For colouring cheese dilute alkaline solution of annatto, which contains nor-bixin, is used. In other countries annatto or ethyl bixinate in oil medium is used for colouring margarine. It is also used for colouring citrus juices, concentrates, drinks, Vienna sausages, candies, etc. Processes are described for colouring cereal-based products, egg noodles and fish, particularly salmon. A browning composition has been prepared from annatto for uncooked food and which can be used also during cooking[19]. A yellow, transparent water soluble ink, stable to heat, hydrogen peroxide and UV light has been prepared from annatto extracts. A hair dye which has antiinflammatory and provitamin A properties has been extracted from annatto seeds. This extract has also been used in cosmetic compositions, shampoos, etc.[20] A 0.1% solution of bixin in ethanol has been used as an adsorption indicator in argentometric titrations. Coloured coating materials have been made with cellulose acetate-phthalate for tablets, pills, granules, etc. An orange red dye has been prepared for use in Kalamkari painting on cotton and wool textiles. This was found to have good colour fastness in washing, sunlight, perspiration testing and pressing[21].

Conclusion

From the foregoing it can be seen that annatto is an important product and the export market for pure bixin is also increasing. It is desirable to encourage commercial plantation of *Bixa orellanna* for domestic use and export.

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Modern Approaches to the Development and Utilization of Plant Products for Pest Management : A Holistic Overview

R.N.Sharma

National Chemical Laboratory
Pune 411 008

As the 20th century draws to a close, environmental concerns and problems have proliferated and accentuated despite increasing technological sophistication. To a large extent, increasing human populations, urbanisation and industrialisation have added to the growing environmental quality degradation. This is particularly noticeable in areas of agriculture and public health where preservation of both have necessitated the deployment of an ever multiplying number of alien chemical molecules the synthetic organic pesticides. In the past few decades, a veritable flood of the latter have inundated the biosphere with consequent gross environmental pollution and resultant ecological havoc. The role of these chemicals in creation of the Green Revolution to meet the bludgeoning demands of food and fiber due to rising demographic pressures is undeniable. Unfortunately equally incontestable are the now very well-documented incontrovertible evidences of a plethora undesirable side effects and serious hazards of the continued extensive, often indiscriminate application of the conventional organic pesticides. These drawbacks and disadvantages extend from immediate and apparent ones of short or long-term toxic and other (carcinogenecity) health hazards to not so apparent but none the less highly significant ones of non-target organism toxicity, persistence leading to biomagnification and general ecological pollution, pest resistance and flareback, secondary pest resurgence, harmful changes in geographical elemental profiles

Pest Management: The New Integrated Philosophy

So phenomenal has been the appreciation of the various negative and controversial attributes of conventional pesticides that there has been a continuing reappraisal of their role as chief bulwarks of Pest Control Programmes. Serious re-evaluation and radical revision of basic levels of Insect Control Philosophy has led to the prominence of the concept of Pest Management by integrated systems incorporating non-insecticidal, or reduced conventional insecticidal deployment[6-10]. Concomitantly, the concept of total pest population eradication, impratical as it was, has been relegated to obsolescence, suppression of pest populations below economic injury levels becoming the new, accepted and achievable desideratum[11,12]. For the realization of the latter, extensive study and research in alternative systems as well as products has been going on round the world over for well over 3 decades now. Apart from discovery and development of non-toxic synthetic molecules such as the different Growth Regulators, considerable attention is also being given to the utilization of feasible/potential derivatives from renewable (chiefly botanical) resources.

Back to Nature : The Plants

This treck back to nature received a good phillip from expositions on the co-evolutionary[13] nature of insect-plant interaction over geological time. It was realized that the virtual 'Arms Race'[14] between these two groups had led to a veritable arsenal of overtly or covertly bioactive products and systems (strategies) which could be used to human advantage by judicious/ingenous manipulation.

In the following sections, occurrence, potential development and classification, underlying philosophical rationales, definitions as well as essential technological parameters for utilization of the most important renewable resource of plants for modern and futuristic Pest Management Programmes are discussed.

Significance : Philosophical Rationales

That anything 'natural' must necessarily be good need not be axiomatic[15]. But to the extent that plant products are naturally evolved ingredients of the biosphere, they have an edge over the alien synthetics, and also rate at least a *priori* environmental suitability. Their co-evolutionary history further makes them ideal sources for entomopathic products.

Small wonder then that there has been a prodigious revival of interest in plant products/chemicals for insect control[16-20]. Painstaking investigations in the past few decades into mechanisms of Host Specificity of insects have yielded considerable insights into the delicate ramifications of insect-plant interrelationships formed over aeons of their co-evolution. These are in a way, significant clues to Nature's own systems of checks and balances which may well be simulated to manage pest

populations. It is now well-established that a special category of chemicals exists in plants. These have been variously called non- nutritional secondary metabolites, token stimulii, *Allelochemics* (the latter term shall be used here). These (allelo) chemicals are distinguished by exercising profound influences on varied aspects of insect biology : behaviour, physiology, growth, reproduction, development, nutrition etc. To a very large extent they determine the success or failure of insects to exploit different host plants. It is obvious that bioactive molecules of such inherent dynamic potential in plants can be suitably exploited (developed) in appropriate strategies of application for biorational and ecocompatible management of insect pest/vector populations. In view of the now incontrovertible high damage/hazard potential of conventional synthetic organic insecticides, the need for developing such environmentally oriented pest management protocols based on the relatively innocuous and scientifically more appropriate botanicals has become imperative.

Occurrence, Classification and Potential

Allelochemics occur in nearly all plants. As clarified earlier, their primary purpose seems to be some kind of interaction with different organisms, viz. insect herbivores (Fig. 4). Recently however, Sharma (1993)[21] has sought to extend such biotic inferences of allelochemics to the hexapod organisation in general. Thus non-herbivorous insects such as omnivorous pests or hemophagic vectors may also be included in the ambit of allelochemics' influence.

From the view point of their entomopathic properties, plant chemicals may be variously classified. One such schemata is presented in (Fig. 1). The first broad category is, of course, of generally toxic compounds which can be made to yield insecticides. The classical examples are nicotine and the rotenoids from the past and the more modern and current pyrethroids. Allelochemics or secondary plant metabolites can be subdivided into Kairomones and Allomones. We define kairomones here as chemicals of adaptive advantage to the recepient organisms i.e. extending 'benefits' to insects. Allomones may be defined as chemicals of adaptive value to the producing/possessing organisms, i.e. affording protection to the plant against the insects. These definitions are logical extensions of literature's and other worker's interpretation which have progressively brought hormones, antifeedants and other plant self defence chemicals[22] into their ambit. There is no need for restriction of these terms to only odorous or volatile compounds capable of emission, as has sometimes been seriously advocated. The revised definitions given here include in the scope of kairomones general or specific attractant + pheromonal analogues or precursors which may serve to attract insect and may thus be useful for population monitoring or selective eradication in combination with suitable insecticide, chemosterilant etc. The allomones cover a broad spectrum of activities such as repellent, feeding and/or oviposition deterrent, hormones, anti-hormones, chitin inhibitors etc. These adversely affect insect behaviour, physiology, development and reproduction and can be used in number of ways for pest management

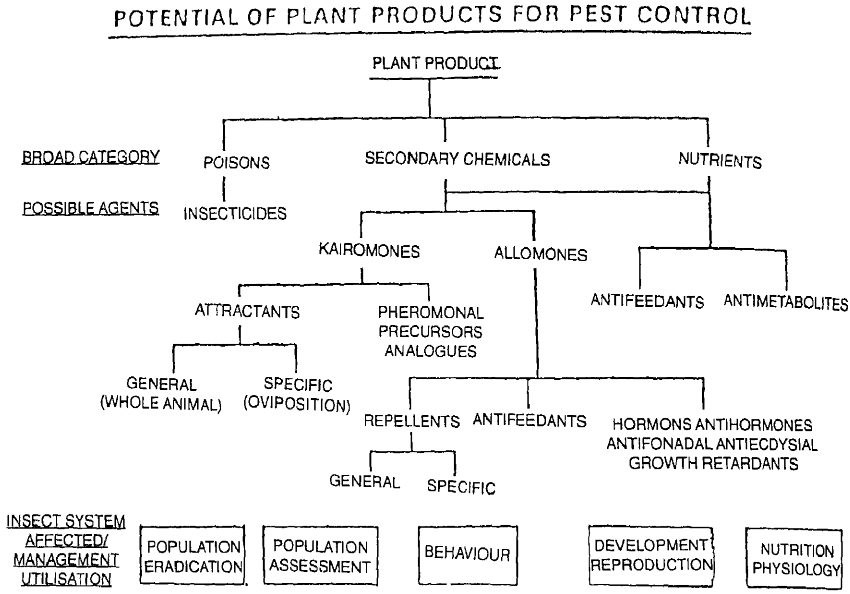


Fig 1 — A broad classification of chemicals obtainable from plants with their effects on insects and possible role in pest management

Systemic antifeedant and antimetabolites are another categories of allelochemics which would deleteriously affect insect nutrition and cause general antibiosis, which may again be utilised in judicious combinations with other principles for insect pest control. Plant products such as lignin, bark, gum, gelatin, saw dust, cellulose, bacterial fermentation products etc. can be used as biodegradable, non-pollutant and slow release carriers or physical agents for pest control in particulate situations. In all cases plant chemicals are used mainly to reduce pollution by substantially restricting the use of toxic products by suitable replacement or application modes ensuring wider, continuous, strictly required and more lasting availability of the latter.

Genesis of the Pest Status in Insects

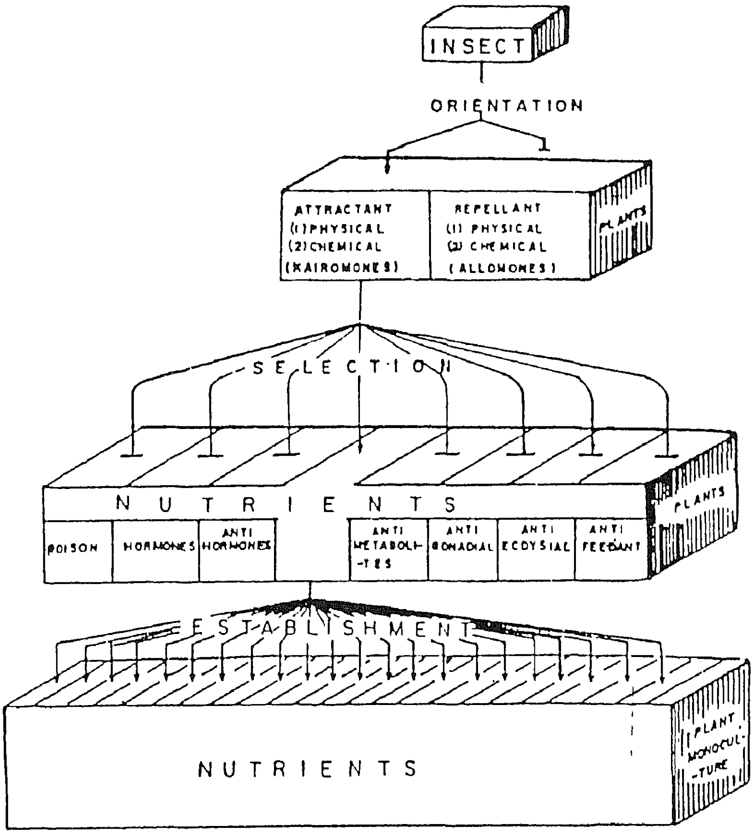
An understanding of the genesis of the pest status for an insect species is important in the design and execution of pest control strategies incorporating the plant chemicals. It may be recalled that of the multitude of known insect species, very few qualify for the epithet "pest"; and of these, an even smaller number merit the

status of "serious" pests. Apparently, evolving without any recognizable immune system such as those of animals, the plants have nevertheless developed sufficient natural adaptations which act as alternative defence systems to protect them from insect attack. An insight into the aetiology of the pest status of the insect can, therefore, identify areas of vulnerability and so help in the formulation of suitable designs for pest control strategies.

In its perennial search for a suitable host plant, a phytophagus insect may encounter a plant either by chance, i.e through random movements, or it may be attracted by physical (colour) and/or chemical attractants (kairomones) Even this first step, in host-selection viz. Orientation, requires the presence of kairomones or at least the absence of allomones such as repellents (Fig. 2) Alightment and stay, followed by feeding, oviposition, larval growth, etc. (i e. Establishment) would again depend on the presence of adequate behavioral stimulants and suitable nutrients and absence of deterrent inhibiting and/or other allomones in the plant. These allomones may affect one or more system of the insect and thus cause antibiosis As such they may be grouped together under the general category of antibiotic principles (Fig 3) which by themselves or in combination with other factors determine the successful establishment or otherwise of the insect on the plant Susceptibility or resistance of a plant to insect(s) is, therefore, also determined by the presence and quality of such principles.

There is some difference of opinion regarding the nutritional adequacy of plants in general to insects One view subscribes[23-26] to the theory that nearly all plants are nutritionally suitable for almost all insects and that establishment of an insect on a plant is governed by the presence or absence of the token stimuli, other allomones (secondary chemicals) in the plant. The opposite viewpoint[20,27-30] holds that apart from secondary chemicals, inadequacy or insufficiency of nutrients can also prevent insect establishment and thus make a plant "resistant" However, without going into the merits of either postulate, importance of the role of the secondary chemicals of plants influencing host selection by insects and, therefore, susceptibility or Resistance of a plant to insect attack cannot be denied. The identification, isolation and development of such compounds can, therefore, be very rewarding and useful for scientific and ecologically suitable Pest Management by their incorporation and utilization in imaginatively designed strategies. Some recent concepts such as the stimulus deterrant, push pull strategy[31] allelochemic-nutrient interactions[32] physiological 'windows'[32] permitting 'enteric odyessies'[33] to allelochemic molecules must also be taken into account in any indepth analyses/value judgements on allelochemic pesticidal potentials.

It can, however, be concluded that presence of a suitable/favourable combination of the allelochemics (especially token stimuli) and nutrients prime the insect for establishment on a plant and the monoculture of human agriculture further abet the irrevocability of the process and development of pest status by providing an unnatural abundance and continuance of the selected host.



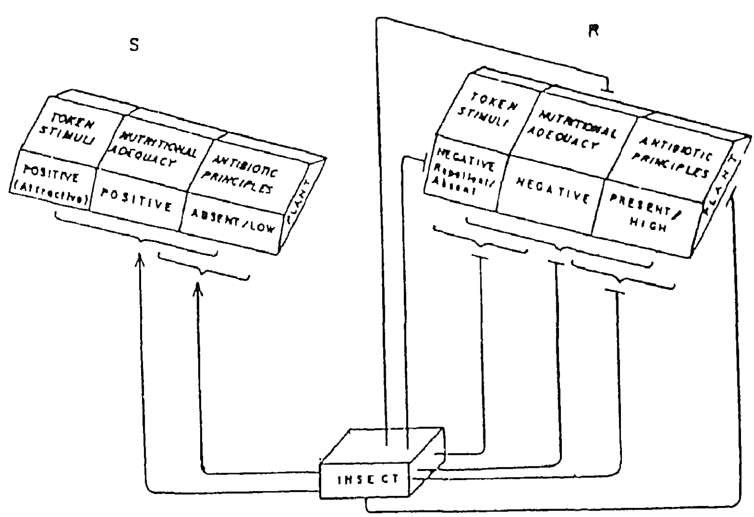


Fig. 2 — Genesis of the pest status in insects

Fig. 3 — The chemical basis of host plant susceptibility (S) or resistance (R)

Methodology

Any programme of screening plants for entomopathic principles needs well-defined objectives and intelligent planning backed by preferably multidisciplinary (biology; entomology; chemistry) expertise and facilities for profitable consummation. The various essential steps of such a programme may be briefly enumerated as follows.

Selection of Plants

Random screening of plants can often be frustrating. Usually leads available in technical literature, folk/one or even heresay sometimes yields rich dividends. Field observations of natural infestation of plants also sometimes provides valuable chemistry. Noxious, undesirable plants such as weeds must always be investigated both for their apparent immunity natural ragage as well as for obtaining some utilitarian advantage from an otherwise undesirable waste. Mention may be made of some recent general[34] or specific[35] compilations listing known bioactivities of plants.

Taxonomic criteria can also be useful. Thus some botanical families are known to be rich in compounds with entomopathic activities. Phenomena such as plant apparency[36] and its various postulates relevant to the present context may also be helpful.

Some examples from our own work illustrate the above. Thus the family Compositae is known to be particularly rich in allelochemics. Similarly the families Lamiaceae and Euphorbiaceae are also fairly rich sources of useful entomopathic chemicals. Among the Compositae[37,38] we found *Parthenium hysterophorus* a classic example combining a number of inviting clues. This plant is a serious weed proliferating unrestrictedly and apparently fairly resistant to serious insect infestations. It is an undesirable weed creating manifold problems of curbing and disposal, apart from its implication in public health and agricultural problems. We were able to trace the resistance of this weed to a potent insect antifeedant principle whose promotion as a pest control agent was, however, not feasible due to its undesirable toxicological side effects. Other allomonic principles in it were not adequate either in quality or quantity to justify further development. The investigation was however successful and rewarding as it conformed to expectations raised from the clues identified, and but for the unfortunate toxicological properties, may have lead to the development of a useful product and profitable utilisation of the weed. Our work with several Lamiaceae plants, and especially *Lavendula gibsonii* has also been similarly rewarding. However, in general entomolgical survey of the vast botanical kingdom is far from adequate and efforts must continue for identifying different sources of potential pest management principles.

Very often it is not possible and/or desirable to examine the whole plant. A decision then has to be made about examination of one or more specific parts. Generally speaking seed, root, inflorescence, leaves and stem, in this order, are the

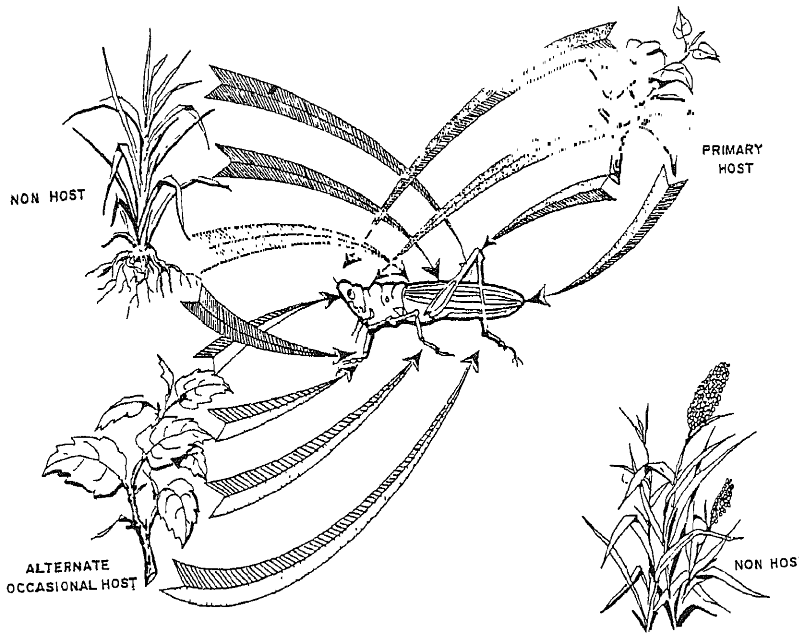


Fig. 4 — (Allelo-)-chemical influences governing insect-plant interrelation

more likely sources of biologically active principles. In the absence of specific leads, however, it may be more rational and convenient to examine whole plant rather than one or more parts separately.

Chemical Procedures

The choice of a solvent or system for the first extraction again poses a dilemma. Thus, a system of solvents of increasing polarity (e.g.petroleum ether, benzene, ethyl ether, chloroform, ethyl acetate,acetone, methanol, etc.) or only one solvent may be used. The choice really depends on the scope and load of work envisaged. It may be mentioned in passing that we have found steam distillates fairly rich sources of especially behaviour manipulating compounds. Since some active principles (e.g. hormonal) may be relatively unstable to heat or photolabile etc., as far as possible all reactions, e.g. extractions, condensations, etc. should be carried out below 40°C in vacuum or under nitrogen and in shade.

If one or other activity is obtained in the total extract with one or different solvents, further follow-up for isolation and identification of the active principles can be a fairly painstaking long drawn out and tedious affair involving successive differential fractionations and biological evaluations of all fractions at every step. The final compounds may be in traces, in combinations or masked by other larger components, thus making their isolation difficult. Sophisticated chemical micro-techniques, GLC, HPLC, esterification, chemical degradation and correlation, synthesis, UV, IR, NMR and mass spectral analysis, X-ray spectroscopy, etc. may have to be resorted to for both isolation of the active principles in pure form and determination of their structures.

Biological Evaluations

These also require considerable forethought, planning design and skill in execution. First, as wide a spectrum of insect pests as possible should be used (Fig. 5). This, of course, depends on the facilities, funds and manpower available. However, attempts should be made to evaluate extractives against atleast 4-5 different pests belonging to different hexapod orders. As far as possible laboratory reared insects

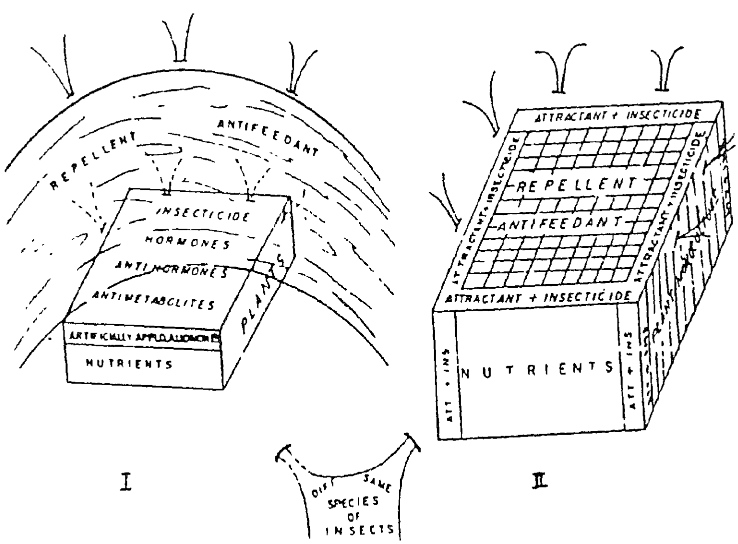


Fig 5 — A comprehensive protocol for development of new pest control agents from renewable resources

of well-defined age, nutrition and physiological status only should be used. Among the dipterans, mosquitoes and flies are both important public health enemies and both are relatively easy to rear in the laboratory. Likewise, the stored products, Coleopteran pests are also easily cultured.At least one or two Lepidopteran pests must also be used since these insects are the real serious pests of agriculture. Hemipteran pests may be useful for assaying hormonal compounds. One or several stages in the life cycle of given pest can be used for the bioassays.

Evaluations of different activities entail different procedures*. The method of exposure to the plant extractive may be by topical application, residual film or through the diet. Much depends on the solubility characteristics of the extractives. Thus, solvents such as acetone, alcohol, etc. which are not themselves toxic to insects can be administered by any or all of the above-mentioned routes. Extracts soluble only in chloroform, benzene, etc can only be applied as surface deposits and care must be taken to ensure complete evaporation of the solvent before the test insects are released for exposure.

For preliminary screenings, as high doses as possible should be used for a fair chance of detection of active principles which may be present in low quantities and masked by other major constituents. However, some limitations exist on the residue application doses in cases of extracts which leave sticky or oily deposits.

Well established parameters[40] and precautions of bioassay procedures such as uniformity of age, physiology, treatment, exposure and post exposure observation period, extensive replication, untreated controls/standards and other checks must all be strictly made/observed to obtain reliable and reproducible results.

Further Development and Promotion

A veritable arsenal of plant based chemicals with potential for useful and effective incorporation in modern biorational integrated pest management protocols already exists. Several comprehensive reviews and books on the properties and status of such compounds are available. However, it may be noted that except for the insecticidal principle (historically the rotenoids, nicotinoids and pyrethroids, and currently the synthetic pyrethrin and the plant based carbamates), only the ubiquitous neem has come to be developed and promoted sufficiently for actual use as a pest control agent. None of the other vast and varied plant principles have so far been developed in a similar manner as economically and technically feasible agricultural products.

* These may sometimes have to be devised de novo and may require considerable ingenuity and skill. Thus, we had to develop our own system of anti-JH, behaviour and antifeedant assays[37-39]

The Concept of Enriched Plant Extractives

This concept was mooted by the author almost a decade ago[41]. The original model for this was the ubiquitous neem plant, extractives and oils of which have been traditionally used in India for routine pest control in food as well as fibre. We developed Neem extractive enriched for particulate biological activity. The author has given the very appropriate name Neemrich, to this extractive[41]. Two enriched extractives were developed viz. Neemrich I having the specific property of oviposition deterrence against the potato tuber moth and Neemrich II with general insect antifeedant and growth regulatory properties. Subsequently, researchers in various parts of world and especially USA, Germany and Israel have been developing various formulations mainly for the terpenoid azadirachtin, known to have the latter two properties. The present status of Neem is that several commercial formulations are being/have been developed with azadirachtin being claimed as the principal active ingredient and its percentage in the formulation serving as a technical standard.

Rider

Based on his work and experience, the author has pointed out that in case of plant extractives, especially from plants like Neem with their known manifold bioactive principles, it is probably misleading to highlight one specific ingredient as the one responsible for the biological effect. In case of Neem, e.g. a number of chemical principles such as nimbin, nimbidin, salanin, nemantriol etc. are known to be present in the extractives. These may reduce or enhance the activity of alleged the major principle (azadirachtin) in Neem. The total biological effect(s) of a plant extractive formulation must be considered attributable to the overall (sum of) chemical constituents. This is borne out by our own studies where sometimes bioactivities of Neem extractives was more compared to pure azadirachtin[42]. In conclusion therefore, it must be stressed that chemical constituents other than the main active ingredient in a plant extractive can not be regarded as neutral excipients in a manner of a conventional synthetic insecticide formulation.

Spiking

The author[21] has also mooted the concept of artificial spiking of enriched plant extractives with particulate ingredients for enhancing bioactivity. Thus, many plant terpenoids, fatty acids, carotenoids, phenols and flavonoids (most allelochemics belong to these categories) are commercially available in abundance and at affordable costs on account of their large scale utility in the pharmaceutical industry. Where active ingredients have been identified, such compounds can be added to plant extractives, or formulations based on their combinations[43] designed for greater effect.

Three Special Case Studies

Neem: The present status of Neem has already been described in the preceding section. It needs to be pointed out further that Neem is probably the first non-biocidal (in the sense of not being acutely toxic) plant extractive to make actual development as commercial field formulation for pest management feasible. Apart from its uniqueness in possessing a host of chemical principles with diverse bioactivity against many insects, neem also has the distinct advantage of being highly ubiquitous and prolific tree in the Asio-African continents. Abundant availability has been mooted by the author as one of the most important pre-requisite for selection of plant for exploiting chemical principles for pest control.

The second case of a plant principle good enough to merit substitution of a synthetic insecticide also comes from our own work[44]. In brief, we found that a plant derivative oil formulated in a special manner (we have given it the name GWINCIL) could effectively destroy cyclops, the vector of dreaded (Naru) guinea worm disease. Currently the latter is sought to be controlled by the use of synthetic organic insecticide, temephos in drinking waters. The disease is endemic in the various parts of the Indian sub-continent and Africa. The author has seen pointing out for long that despite the WHO's clean chit for the use of temephos in drinking water, considering the nutritional, general hygiene and health status of populations affected by the guinea worm disease, subjecting them to fruther chemical abuse of an organophosphate insecticide in their drinking water must be viewed with serious reservations. Replacement of temephos by a relatively innocuous plant derivative such as GWINCIL developed by us therefore deserves serious consideration.

The third illustrative example is again from our own work on *Heteropsylla cubana*[46], a newly emerging pest of social forestry in Asia. For various reasons, it is not possible to use conventional synthetic insecticides in social forestry. We were able to identify several plant derivatives which could be used for controlling this pest.

It may be noted that of the three very definitely promising cases cited above, only Neem has so far actually been commercially exploited. The various reasons for the generally disappointing outlook for field transmission/commercialisation of plant products are discussed in the following section.

Problems

There are several reasons why more promising potential pest control agents from plants have not been able to replace the established conventional insecticides. First, it is difficult to overcome the bias in favour of the latter due to their easy availability, simple application and misconceptions about their efficacy (e.g. quick dramatic results such as overkill and persistence, etc.). In contrast, no system or methodology has been evolved for the successful transfer of the candidate plant based pest control agents from the laboratory to the field. Again certain inherent properties of plant

products, although scientifically and ecologically desirable, are likely to be misinterpreted as limitations by the actual users. Thus, plant products are likely to be less persistent due to inherent instability or biodegradability, and their activity may extend to only few or even one pest species. Both these properties are in conformity with modern concepts of pest management. However, the need for repeated applications and devising ingeneous combinations involving more than one product for greater efficacy, not to mention the misleading subtlety of the results spread over in time, all combine to create misconceptions and lack of confidence in the layman user. Again, active plant principles are likely to be complex molecules whose analogue synthesis and commercial/ industrial development as a product may be economically or otherwise not feasible.

In the ultimate analysis at the present juncture, it is the overall profitability of any agrochemical product which shall determine its viability. It has been possible after many years of research as well as increasing pressure from environmentalists to procure a limited amount of support for the actualisation of Neem as a field product. Its technical and economic viability will undoubtedly affect the future of plant products in general.

Solutions and Advantages

We have seen that biodegradability, limited residual activity and range (specificity), far from being objectionable, can instead be highly desirable and important features of the hypothetical ideal pest control agent model as it is conceived today. Further, increasing sophistication of chemical technology in recent years promises, development of smaller, simpler, better and technically and economically feasible compounds based on the more complex plant models, where necessary. Where crude extracts themselves are sufficiently potent (e.g. Neem extractives), low costs and simplicity of production, combined of course, with their general non-hazardous character, can nullify any disadvantages of repeated applications.

The crux of the difficulty in developing and promoting plant products, however, lies in the absence of any concerted attempts to evolve a system of technology transfer such as is established for the conventional insecticides. Such a system can be developed for plant products also. Thus, potential pest control agents from natural sources can be:

1. Developed as patented processes/synthetic products in the organized industrial sector. Here too, as with many conventional insecticides, benefits to the farmer would depend largely on the extent of Govt. subsidies:
2. Promoted as cheap, safe, effective and above all environmentally acceptable pest control agents. This can generate jobs at the production level by way of cottage industry/individual enterprise. Further job opportunities would also arise for the rural literate in extension services. The latter should be considered an essential pre-requisite. An infrastructure aiming at a nominal one extension

worker per group of five to ten villages must be built-up to provide necessary technical information for production as well as use of such products, not to mention proper publicity for popularization leading to consumer acceptance.

On account of little or doubtful possibilities of substantial pecuniary gain, it would be futile to expect the Pesticide Industry (Private Sector) to take any initiative in the development and promotion of plant products. However, Agroindustrial Corporations, Agricultural Universities, Rural Development Centres, Village Panchayat Samities, etc. must be encouraged to play an effective role in the dissemination of the proper perspective and in promoting the production and use of plant products in the fields.

The precise role and mode of operation of such bodies should be examined and defined and definite guidelines established to enable smooth and successful transfer of this technology from the research scientist to the lay agriculturist.

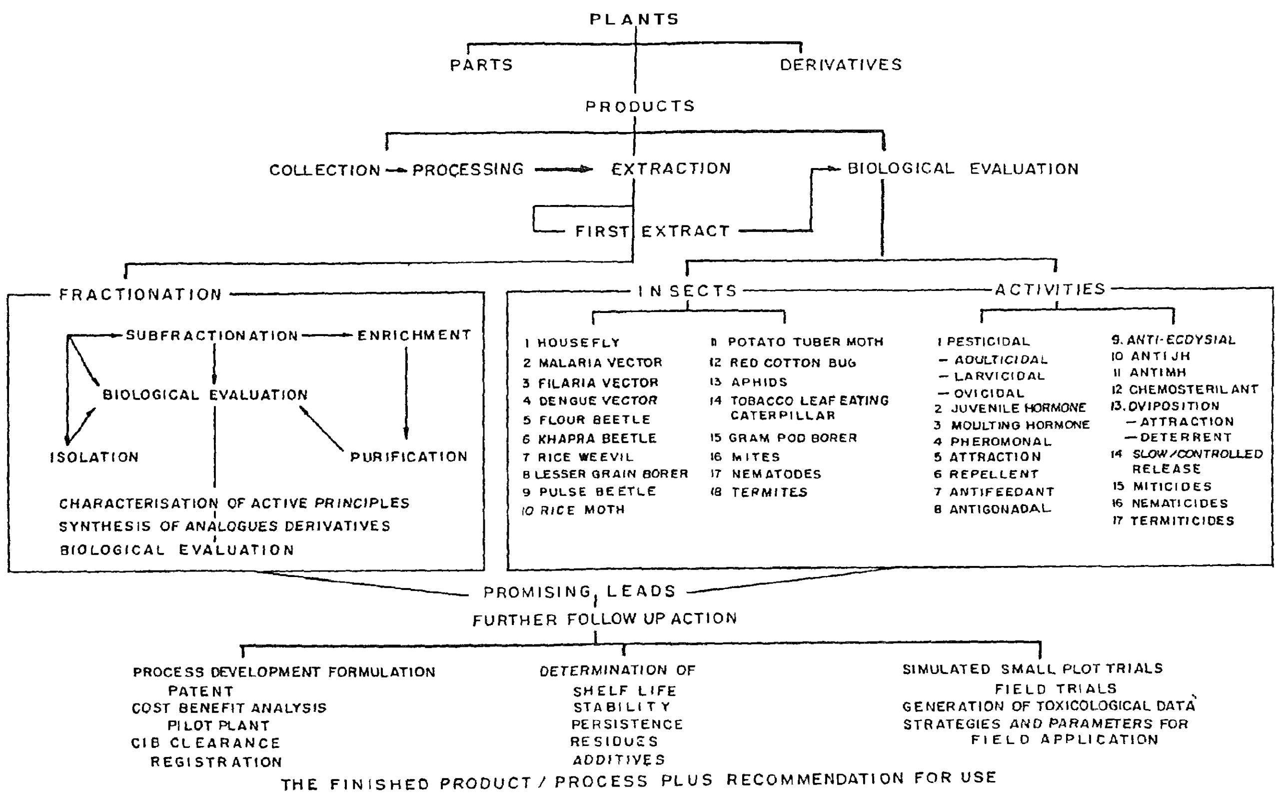
Finally, attention must be drawn to a simple arithmatic. Pest control by conventional insecticides carries with it tangible and intangible cost and risk to the environment, food, livestock and human health. It becomes highly debatable whether insect control by conventional methods is indeed 'economical proposition' is viewed in context of the associated other costs. It may, therefore, well be argued that incorporation of plant products in pest management programmes as supplement or exclusively would probably be more economical by elimination of 'other' costs indicated above. It only requires a broader overall overview of the entire situation rather than the narrow parameter of specific pest eradication only, as exercised currently.

New Strategies

Once a system has been established to ensure the influx of a large number of plant products for use in the field, new strategies deploying judicious combinations can be devised by entomologist, field tested and passed on to the agriculturist through the extension networks. Thus, assuming low cost and no risk character of the plant products, generous and repeat applications can be permitted. In Fig. 6, two such hypothetical strategies are depicted. Strategy 1 (Fig. 6) envisages a generous repellent and/or antifeedant cover, further supplemented with a suitable combination of artificially applied Allomones. Strategy II combines peripheral use of appropriate attractant with insecticide and further precautionary protection by liberal use of repellents and/or antifeedants on the crop itself. In both these hypothetical schemes, liberal and repeated applications of the various natural pest control agents should be possible due to their low or negligible cost and risk.

Specific Areas of Research

Areas of research which can be identified in light of available information would include development of the following categories of compounds from plant sources:


PLANTS
PARTS
DERIVATIVES
PRODUCTS
COLLECTION
PROCESSING
EXTRACTION
BIOLOGICAL EVALUATION
FIRST EXTRACT
FRACTIONATION
SUBFRACTIONATION
ENRICHMENT
BIOLOGICAL EVALUATION
ISOLATION
PURIFICATION
CHARACTERISATION OF ACTIVE PRINCIPLES
SYNTHESIS OF ANALOGUES DERIVATIVES
BIOLOGICAL EVALUATION
INSECTS
1 HOUSEFLY
2 MALARIA VECTOR
3 FILARIA VECTOR
4 DENGUE VECTOR
5 FLOUR BEETLE
6 KHAPRA BEETLE
7 RICE WEEVIL
8 LESSER GRAIN BORER
9 PULSE BEETLE
10 RICE MOTH
11 POTATO TUBER MOTH
12 RED COTTON BUG
13 APHIDS
14 TOBACCO LEAF EATING CATERPILLAR
15 GRAM POD BORER
16 MITES
17 NEMATODES
18 TERMITES
ACTIVITIES
1 PESTICIDAL
– ADULTICIDAL
– LARVICIDAL
– OVICIDAL
2 JUVENILE HORMONE
3 MOULTING HORMONE
4 PHEROMONAL
5 ATTRACTION
6 REPELLENT
7 ANTIFEEDANT
8 ANTIGONADAL
9. ANTI-ECDYSIAL
10 ANTI JH
11 ANTIMH
12 CHEMOSTERILANT
13. OVIPOSITION
– ATTRACTION
– DETERRENT
14 SLOW/CONTROLLED RELEASE
15 MITICIDES
16 NEMATICIDES
17 TERMITICIDES
PROMISING LEADS
FURTHER FOLLOW UP ACTION
PROCESS DEVELOPMENT FORMULATION
PATENT
COST BENEFIT ANALYSIS
PILOT PLANT
CIB CLEARANCE
REGISTRATION
DETERMINATION OF
SHELF LIFE
STABILITY
PERSISTENCE
RESIDUES
ADDITIVES
SIMULATED SMALL PLOT TRIALS
FIELD TRIALS
GENERATION OF TOXICOLOGICAL DATA
STRATEGIES AND PARAMETERS FOR
FIELD APPLICATION
THE FINISHED PRODUCT / PROCESS PLUS RECOMMENDATION FOR USE

1. Pesticidal chemicals such as the pyrethroids; other toxic agents and their analgoues.
2. Antifeedants.
3. Pheromonal analogues/attractants/repellents.
4. Hormonal and antihormonal analogues.
5. Chitin (ecdysial) inhibitors.
6. Natural products for development of slow release formulations of pest control agents.
7. Using Biotechnology for development of transgenic plants.

Conclusion

Conservation of environmental quality afforded by the type of natural products described in this article is more than sufficient justification for continuing and increasing inputs of enthusiasm, efforts and funds for their development, adoption and promotion. In light of modern perceptions, the environmental advantage gained through the successful replacement of drastic reduction of any one conventional synthetic organic insecticide by such products is immeasurable and overides any pecuniary consideration. Also, as analysed here, plan development and promotion of plant products for insect control can yield diverse returns by opening avenues of individual/small-scale cooperative/cottage enterprises and generate job opportunities in especially the rural/agricultural sectors.

The need for continuing concerted and dedicated efforts for the development and adoption of natural pest control agents can hardly be overemphasised. Where as in the decade since the first appearance of this article, environmental awareness has definitely increased, the money and the political power of the insecticide lobby in India has certainly not decreased. The environmental lobby on the other hand continues to remain largely disorganised. Unfortunately too, the so called environmentalists are commonly generalists instead of experts as for example in entomology, biology or chemistry. It is therefore difficult to make or sustain arguments and policies and force their acceptance in the national decision making bodies. It is singularly unfortunate that even the more enlightened segments of our community, including the specialists who should know better continue to ignore the various warning signals which have surfaced in recent years. There is also a distinct propensity to treat the various objections against conventional insecticides as a fad of the west or dismiss them on grounds of lack of resources to take corrective action. It has already been pointed out in this article that such reasoning is fallacious. The various corollaries of conventional insecticide use e.g. insect resistance, pest flareback, secondary pest resurgence etc., cause enormous damage to natural resources and the environment. This coupled with hazards to human created by the phenomena of biomagnification and chronic toxicity characteristic of the conven-

tional pesticides, make the latters 'costs' far outweigh any benefits. Taking into account all the tangible and intangible disadvantages, the use of conventional insecticides can hardly be called 'control' if the latter term implies a positive economic gain.

It has been more than apparent for quite some time that the indiscriminate and undiminished use of conventional synthetic organic insecticides has been badly, perhaps irretrievably mutilating all our eco-systems. To meet the ever growing needs of both public health and agriculture and at the same time prevent further degradation of the overall environmental quality, it is imperative to seek, develop, adopt and promote the newer type of pest control agents from natural sources. In the long run they are our only hope of redemption from the unenviable situation we are in.

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*NEEM (*Azadirachta indica* A. Juss.) As a Source of Pest Control Material

B. S. Attri

Ministry of Environment and Forests
CGO Complex, Lodhi Road
New Delhi - 110003

Introduction

NEEM (*Azadirachta indica* A. Juss.) is a large evergreen tree which is native to Indian sub-continent It grows in almost all parts of India except under continuously low temperature conditions as in the upper Himalayas. It is also observed to avoid areas of high rainfall. After revelation of its unique medicinal and other uses over the past century, the neem has been introduced in other south-east Asian countries, tropical Australia, Africa, central and south America, Fiji, Papua New Guinea, Brunei, Phillipines, Mauritius, the Caribbean, Puerto Rico, the Virgin Islands, Sudan, Niger etc. (Saxena, 1993). Recently it has been introduced in Saudi Arabia (Ahmed *et. al.,* 1989).

Ketkar (1976) reported that the total number of neem trees in India was estimated around 14 million, neem oil around 83,000 tonnes and neem cake about 3,30,000 tonnes pa. Since neem got considerable attention during the past 2 decades in India, the present figures are expected to be higher. Singh and Kataria (1993) put the number of trees around 19 million.

Use of neem to ward off damage by pests is known since antiquity. Ancient Indian folk mixed dried neem leaves with grain meant for storage. Interest in neem

research for pest control was triggered after the health hazards and adverse ecological effects of chemical pesticides were recognised. It was in 1962 when Pradhan and his associates first time discovered the antifeedant action of neem seed extracts to locusts. Since then lot of literature has been published on its pecticidal properties and other useful attributes for medication and health care. Recent two publications on "Neem Research and Development" (Eds. Randhawa and Parmar, 1993) and "Botanical Pesticides in Integrated Pest Management" (Eds. Chari and Ramaprasad, 1993) cover various aspects of neem namely, botany, chemistry, genetic diversity, bioactivity, use in health care and medication toxicology etc. This article reports recent advances in neem research for pest management, neem-based products that have been developed and some promising areas for future research.

Chemistry of Known Pesticidal Molecules in Neem

Chemistry of neem has been recently dealt by Devakumar and Sukhdev (1993). The bioactive compounds in neem can be classified into 2 categories viz. isoprenoids and others. The latter comprise glycerides, polysaccharides, sulphurous compounds, flavonoids and their glycosides, amino acids etc.

Siddiqui (1942) first isolated 3 bitter principles namely, nimbin, nimbidin and nimbinin from its oil. Several new compounds were discovered later. Henderson *et al.*, (1964) reported Salamin, a terpenoid possessing feeding deterrent activity. Another compound, Meliantriol, was identified by Levie *et al.*, (1967) which had similar property. More than 50 compounds with anti-pest activity have been identified from various parts of neem tree (Jones *et al.*, 1990) and still many more remain unknown.

Among the various compounds known so far, *Azadirachtin* reported by Butterworth and Morgan (1968) is the most powerful which possesses not only feeding deterrency but also growth disrupting potential. Moreover, its yield is the highest making it attractive for practical use. The chemical structure of azadirachtin remained controversial till Broughton *et al.*, (1986) through X-Ray crystallography reported it as given in Fig. 1. Future research on identification of bioactive compounds is expected to be rewarding.

Pest Control Uses

Neem products have been tested for various properties against more than 300 species of insects, throughout the world. In India, it has been evaluated against 106 species of insects, 12 species of nematodes and atleast 9 species of fungi (Singh, 1993a, 1993b). Among insects the maximum number is from Lepidoptera (32) followed by Coleoptera (28), Hemiptera (23), Diptera (12), Orthoptera (6), Thysanoptera (2), Hymenoptera (1), Siphonaptera (1) and Isoptera (1). Neem products could be used as:

(1) Antifeedant
(2) Repellent
(3) Insecticide
(4) Growth disruptor

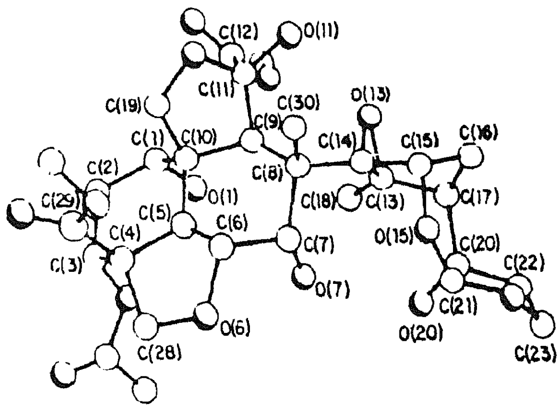

Fig. 1–Molecular structure of azadirachtin (Broughton, *et al.*, 1986)

(5) Oviposition deterrent
(6) Antifecundity agent
(7) Attractant
(8) Nematicide
(9) Anti-microbial

Antifeedant

The most pronounced effect of neem derivatives against insects is their antifeeding effect, which is often indistinguishable from repelling effect. Feeding deterrency was first recorded by Mann and Burns (1927) who observed uniqueness of *Melia azardirachta* not being eaten by desert locusts (*Schistocerca gregaria*). Clear presence of feeding deterrent in neem was first demonstrated by Pradhan *et al.*, (1962) to desert locust (*S. gregaria*) and migratory locust (*Locusta migratoria*) at the Indian Agricultural Research Institute, New Delhi. As low as 0.1% fresh neem seed powder mixed in water was able to provide complete protection to the treated foliage under field conditions. Since then it forms a standard and most reliable recommendation for protection of agricultural crops from locust invasions.

Antifeeding effect of neem derivatives varies with the insect species, stage of growth of the test insect and the dose. Most insects can be deterred from feeding by less than 0.5% neem seed suspension in water, though some may require as high as 3%. Neem oil has negligible antifeeding effect and hence requires high concentrations. It has been demonstrated that 12% neem oil emulsion when sprayed on paddy suppresses/avoids feeding by brown plant hopper *Nilaparvata lugen* Stal. (Saxena *et al.*, 1981). However, such a high dose makes the findings less exciting.

Based on bioassay tests, it has been concluded that the active principles can be best extracted in ethanol followed by water (Singh, 1987). Free solubility in water

meant that home made highly effective formulation can be easily prepared with water. This should be reckoned as very valuable observation to take safe plant protection to the poor rural masses with their still crude methods of farming.

Effect of storage on the antifeedant activity is not fully studied. It is, however, known that the activity deteriorates faster in powdered seed than in whole seeds (Attri and Singh, 1977).

Limited systemic action of neem derivatives has been reported. This has been demonstrated by Gill and Lewis (1971) against *S. gregaria* and by Saxena *et al.*, (1984) on *Nephotettix virescens* and *Nilaparvata lugens* using neem seed cake in paddy fields.

Repellent

Neem contains several aromatic and adorescent compounds which repel insects. Often antifeeding and repellent effects are indistinguishable. Experiments conducted on the use of neem products on stored grain or on crops largely do not indicate whether the relief was due to repellent or antifeedant action. In spite of this, clear repellent effects have been observed. Saxena (1993) observed that even without contact, paddy crop sprayed with neem oil repelled plant hoppers *N. lugens* and *Sogatella furcifera*. It has also been observed that white ants (*Microtermes* sp.) and some species of ants do not like the neem scent and move away from the treated vicinity. Neem cake has been used since long in Madhya Pradesh for clearing termite-infested areas. Further studies on this line and on oviposition by insects could be rewarding.

Insecticide

Insecticidal activity as discussed here refers strictly to contact action or immediate stomach toxicity and excludes death of insects due to various other effects of rather latent nature. By and large neem has weak insecticidal activity even in its purified compounds like azadirachtin. Water suspension of NSK was reported toxic to soft-bodied insects like aphids (Cherian and Menon, 1944; Goyal *et al.*, 1971, Singhm *et al.*, 1988).

'Neem oil extractives', a waste from neem oil refining has been found to be effective mosquito larvicide (Attri and Ravi Prasad, 1980). The material acts as instant killer of the first instar larvae of *Culex fatigans* at 0.04% concentration whereas at lower concentrations it had delayed toxicity. As low as 0.005% neem oil extractive was effective in causing 100% mortality of the Ist stage larvae before they could emerge as adults. Delayed toxicity as described here may be due to mere starvation or some unknown physiological reasons. The efficacy as observed here has opened an outlet for remunerative disposal of this waste product. Incidentally, it has been found to be safe to natural enemies of mosquito larvae like *Gambusia* fish and Tadpoles at normal dosage.

Growth Disruptor

It is said that prevention is better than cure. If we can check the built up of insect population, there would be no need for applying any chemical control methods which often cause adverse ecological effects. Neem derivatives have been demonstrated to possess lethal effects on insects by upsetting the natural harmonal balance causing growth disruption. These effects include prolonging/reducing the normal moulting period in larvae causing death even before pupating or during pupation. Similar effects in nymphal moults as in larval moults in case of those insects which do not pupate, emergence of abnormal forms such as larvae, pupal or pupal adult intermediates or miniature adults with deformed reproductive organs and reduced life span. These latent effects cause progressive decline in insect population leading to its total inhilation in due course of time. In fact, neem should be credited for this silent pest control property.

Growth disrupting activity of neem was first reported by McMillan *et al.*, (1969) on *Spodoptera frugiperda* and *Heliothis zea* with chloroform extract of leaves of *Melia azedarach*, a close relative of neem. Growth disrupting effects of extracts of neem seed kernel were later reported on *Pieris brassicae* (Gill and Lewis, 1971), *Epilachna varivestis, Leptinotarsa decemlineata* and *Plutella xylostella* (Steets, 1976), *Culex fatigans* (Singh, 1984) and *C. pipiens* (Zebitz, 1987). Among other derivatives, neem oil is known to interfere in normal growth processes of insects. Saxena (1987) reported ecdysal failures and other development deformities on rice bug, *Leptocorisa oratorius*.

Neem leaves, seed kernel powder, cake and oil when mixed with stored grains protected them from damage by inhibiting growth, development and emergence of adults.

Precise studies on insect growth regulatory effect have been conducted using azadirachtin (Gujar and Mehrotra, 1984; Koul, 1985). The mode of action of azadirachtin is not yet clear. It is known to reduce and delay titre of morphogenic hormones. It is also suggested to be interfering with neuro- endocrine system which controls ecdysone and juvenile hormones (Kubo and Klocke, 1986). More investigations are required to elucidate the mechanism of its action.

Ovicide

Ovicidal effect of neem derivatives is little known. It was reported that Trialeurodes eggs laid on the leaves of brussel sprouts sprayed with 1% neem seed suspension either did not hatch or the larvae died in egg shells partially hatched. Similarly eggs of *Stomoxys* incubated on filter paper moistened with 0.01% azadirachtin did not hatch (Gill, 1972). These studies indicate that growth disrupting factor penetrated the egg shell and affected the developing embryo. This should be considered as a very valuable observation for the practical pest management and deserves extensive research.

Oviposition Deterrent

Neem contains a variety of chemicals which interfere in normal mating behaviour of insects and may deter them from laying eggs on the treated surface. Disruption of normal courtship signals and mating behaviour has been reported on *N. lugens* when caged on plants sprayed with 3% neem oil emulsion (Saxena *et al.*, 1989a). At higher concentration of oil, the females did not call at all and consequently the males could not locate the females. A few females that emitted signals were not in tune with male calls and the duration of the calls were decreased significantly. Neem oil deterred egg laying by *N. lugens* (Saxena *et al.*, 1981). *Amrasca devastans* (Saxena and Basit, 1982), *Bemesia tobaci* (Coudriet *et al.*, 1985) and pulse beetle, *Callosobruchus* sp. (Yadav, 1985). Extract of neem seed kernel caused 90% oviposition deterrency of *Heliothis armigera* on chick pea under laboratory conditions (Jhansi Rani, 1988).

Antifecundity Effect

Neem products are known to reduce fecundity and hatchability of insect eggs (Schmutterer, 1990). This has been reported in case of rice green hopper, *Nephotettix virescens* (Abdul Kareem, 1988), cucurbit fruit fly, *Dacus cucurbitae* and *D. dorsalis* (Singh and Srivastava, 1983). Drastic reduction in the fecundity was observed in *Spodoptera litura* following azadirachtin treatment. Untreated moths laid as many as 7,923 eggs whereas only 705 eggs were laid by 5 pairs of azadirachtin treated moths, the reduction being almost 90% (Gujar and Mehrotra, 1984). This property has a great role to play in gradual, rather invisible, reduction of insect population in practical pest control.

Attractant

Neem leaves attract the adults of white grub (*Holotrichia* sp.) which are serious leaf defoliators of several tree species. The grubs damage tubers and roots of several crops. Neem has been successfully utilised for control of this pest (Gupta, 1973; Khan and Ghai, 1974). Neem twigs with leaves are inserted in rows in the infested pockets around sunset time at the start of monsoon. At dust, swarms of these beetles which normally come out to feed on foliage of various plants, are attracted to these twigs where these are mechanically killed. The nature of the attractant is not yet known.

Nematicide

The potential of neem for the control of plant parasitic nematodes has been exhaustively reviewed recently by Alam (1993). Only some relevant information is included here.

Utility of neem and its derivatives for nematode control has now been established beyond doubt for management of nematode problem. Among different neem materials, neem cake has proved to be the most effective in controlling various nematode species. In addition, it has several merits over synthetic nematicides, none

of which could gain popularity for large scale use. Neem cake is eco-friendly product in true sense. It is biodegradable, plant-based, non-polluting, nutritionally rich manure which improves soil structure and fertility, and is ecologically safe. It is remarkably cheap and easy to apply uniformly in the soil. Hence, neem cake is the finest material known today for practical control of nematodes.

In addition to neem cake, the leaves (Akhtar and Alam, 1991; the root (Alam *et al.*, 1977). The flower, the bark and the gum (Siddiqui and Alam, 1985) have also been reported to possess nematicidal properties. The nematicidal effect of neem cake often lasts for 2 cropping seasons.

Effectiveness of neem cake has been found to be better or at least comparable to synthetic nematicides (Akhtar and Alam, 1991; Ahmad *et al.*, 1990). Seed treatment with synthetic systemic nematicides before sowing in the soil treated with neem cake has proved still more effective. For example, treatment of seeds of mung and lentil with fensulfothion and phorate respectively and then sown in soil amended with neem cake was found to be highly effective method for control of plant parasitic nematodes (Mishra and Gaur, 1989; Gaur and Mishra, 1989).

It has been observed that application of N, P and K alone as fertilizer to soil results in marked increase of plant parasitic nematodes. But if applied in combination with neem cake, the population dwindles slowly and steadily (Gaur and Prasad, 1970).

Neem possesses a variety of chemicals and it is not difficult to imagine that the crude neem derivatives will exert their effect in several ways. Based on the data and theories put up to explain that the mode of action by various scientists, Alam and Jairajpuri (1990) have summed up it as below:

1. Change in the physical and chemical properties of soil inimical to nematodes
2. Release of plant nutrients which accelerate rapid root development and overall plant growth thus helping the plant to escape nematode attack.
3. Induction of resistance/tolerance in plant roots against plant-parasitic nematodes.
4. Nemato-toxic substances present in the amendments are released after dissolving in water.
5. Increase in predaceous and parasitic activity of soil biota.
6. Toxicants are produced during microbial decomposition.
7. Metabolites of microbes which become active during decomposition of organic amendments are toxic to plant-parasitic nematodes.

Anti-pathogen/Anti-microbial

Neem is already well known to possess curing property for skin and viral infections of human beings and animals. This has now been checked on plants. The information available has been reviewed recently by Parveen and Alam (1993). It is now

well established that the neem possesses some fungicidal and antiviral potential. The growth of *Fusarium oxysporum* f. sp. *ciceri, Rhizoctonia solani*, and some other soil-borne fungal pathogens of chickpea (*Cicer arietinum*) was inhibited in the liquid medium containing extracts of leaf, trunk, bark, fruit pulp and oil of neem (Singh *et al.*, 1980). Chickpea seeds treated with neem oil sown in soil infested with pathogens produced disease-free seedlings, whereas all the seedlings from untreated seeds exhibited disease symptom.

Leaf extract of neem has been reported to successfully control the fruit rot disease of cucurbitaceous plants (Krishna *et al.*, 1986) and fungal diseases of groundnut (Bansal and Sobti, 1990 ; Ghewande, 1989).

Neem oil 1% and 10% emulsion sprays were able to inhibit the growth of *Alternaria alternata* by 61% and 100%, respectively (Vir and Sharma, 1985). Neem seed cake (2%) reduced muskmelon wilt caused by *Fusarium solanii* by 80% (Chakrabarti and Sen, 1991). Pant *et. al.*, (1986) reported that some sulphur compounds present in neem leaves possess the reported fungicidal activity.

Attention is drawn here to our failure in controlling fungal diseases that infest the crops virulently during rainy season. Attempts to control by spraying chemical fungicides are almost infructuous as the chemical is quickly washed away by repeated rains. Neem oil, if effective, holds great promise under such circumstances. Late potato blight spread by *Phytophthora infestans* and apple scab are cases in view. Both these diseases cost millions of rupees to potato and apple growers and still without much success. Neem oil is worth testing in such cases.

Anti-viral property of some neem derivatives has been reported. The anti-viral effect is either through inhibitng the virus growth or indirectly by killing the insect vector. Verma (1974) reported that nimbidin and nimbin, obtained from neem oil were active against potato virus. Inhibitory effects of extracts of neem were found on spinach mosaic virus on the host *Chenopodium amaranticolor* (Zaidi *et al.*, 1988; Singh *et al.*, (1988a). There are several reports where use of neem derivatives has reduced the viral infection by controlling the insect vector particularly on rice (Srivastava *et al.*, 1986; Mariappan *et al.*, 1987).

There is practically no information on the use of neem against plant pathogenic bacteria. Nevertheless, there is a great need and scope for work and optimism for success in this line.

Inhibition of pesticide degradation in soil has been reported by Ravi Prasad (1979). This finding provides a clue to develop insecticide granules with regulated release by using neem derivatives or other antimicrobial agents, an entirely new approach.

Toxicological and Ecological Effects

Effects on man: Neem derivatives except neem oil, are practically non-toxic. Alcohol and aqueous extracts of seeds have no oral or dermal toxicity to mammals. Sodium nimbidinate did not produce any local or general side effect on human beings. (Bhide *et al.*, 1988). However, neem oil toxicity has been reported. It produces symptoms like vomitting, diarrhoea, nausea and general discomfort (Chopra *et al.*, 1965). In Malaysia acute poisoning of over a dozen infants was reported after injestion of 5ml of neem oil (Sinniah and Baskaran, 1981). Another death of a 4 months infant given 12 ml of margosa oil for 2 successive days has been reported (Sinniah *et al.*, 1982). It was, however, suspected that contamination of oil with aflatoxin might have synergised the toxicity. There are reports of renal failure in Ghanians who were drinking leaf tea for malarial treatment (Kunungo, 1993). It is hence concluded that neem oil in particular needs to be used with proper care and precautions, though its toxicity and per se is negligible as compared to synthetic pesticides.

Ecological Effects: Neem products are ecologically safe at doses much above the normally used. Higher doses have, however, been reported to be toxic in some cases particularly to the aquatic life. Neem oil extractive at 0.005% was non-toxic to insectivorous fish *Gambusia* sp. but caused 100% kill at 0.04% concentration. This concentration (0.04%) was also toxic to tadpoles causing 80% mortality in 24 hours and 100% in 2 days. However, this material was non-toxic at 0.01% concentration (Attri and Prasad, 1980). Margosan-0 is also reported to have mild toxicity to rainbow traut and bluegill sun fish but has been reported to be benign to honey bees at well above the recommended doses of 20 ppm foliar spray (National Res. Council, 1992).

Avian toxicity studies have been carried on poultry birds by feeding them water extract of neem seed kernels. This caused severe hepatitis and mild to severe nephritis (Verma, 1974). Interestingly, neem berries are favourite fruit of certain tropical birds in Nigeria and Kenya (Doria, 1981).

The above studies indicate that neem products need to be used with due care in aquatic bodies whereas these are reasonably safe for use in other ecosystems.

Neem in Partical Pest Control

As discussed in preceding section, the neem possesses about a dozen pest control properties and the neem products are ecologically safe. In spite of this, it has not yet been widely adopted for pest control. The main reaons for its low profile are:

(i) Most entomologists have evaluated the success of neem derivatives on the basis of their instant killing ability vis-a-vis that of synthetic chemicals. It is well known that neem is a weak instant killer for most of insect species. It rather acts slowly over long periods by exerting its other effects like deterring the insects from feeding,

discouraging egg laying, disrupting normal growth etc. so that ultimately the population declines but steadily. This has not been adequately appreciated. Long-term studies on the population dynamics with repeated application of the test material are required to assess the real success.

Integrated pest management aims ultimately at saving the crop from pest ravages and increasing the yield. The assessments carried so far did not often go into in-depth cost-benefit analysis which take into account the advantages accrued due to repellency, feeding deterrency, overall reduction in population, enhanced crop growth (due to application of materials like neem cake which serves as nitrogen regulator and excellent manure) plus the ecological benefits. Ignorance of all these factors have put other products at unfair advantage over neem products.

(ii) ***Lack of Suitable Commercial Formulations***: This is the age of readymade goods now. People have little patience to collect, process and preserve neem materials and prepare the formulations themselves. There is a need for commercial formulations based on neem derivatives. Recently some formulations have been put up in the market, which are struggling to make a place for themselves. Main problem with these formulations is their short shelf-life. All plant products tend to degrade with time unless properly formulated. Not only that, the material when applied on the crop is susceptible to thermal and photodegradation and later to microbial degradation. Hence formulation technology assumes special significance.

(iii) ***Technology Transfer***: No technology becomes popular unless adequately demonstrated. Hardly any training programme is arranged by the State Agriculure Deptts. or Central Govt. Agencies for repairing simple yet effective formulations from neem at farm level. Publicity and demonstrations on TV are totally ignored. Govt. incentives are rare. Under such a scenario, a strong push is required for technology transfer by all the agencies involved.

With all this, environmental problems associated with use of chemical pesticides have created special interest in neem products. Considerable work has been recently done to use neem derivatives either alone or in combination with other methods of control for management of various pests of rice, cotton, groundnut, tobacco and vegetables as well as mosquito in malaria control. These are the cases where significantly large quantities of pesticides are used and still without much success.

On rice, field experiments have demonstrated reasonably good control of gall midge, *Orseolia oryzae* W-M (Samalo *et. al.*, 1990), leaf folder, *Cnaphalocrocis medinalis* (Rajasekaran *et. al.*, 1987; Krishnaiah *et al.*, 1990), brown leaf hopper, *Nilaparvata lugens* and green leaf hopper, *Nephotettix virescens* (Saxena, 1989b), earhead bug, *Leptocoriza oratorius* (Raguraman, 1987), stem borer, *Scirpophaga incertulas* (Singh *et al.*, 1990). On cotton, neem oil emulsion and neem seed or

neem cake extract have been found to give satisfactory control of cotton white fly, *Bemesia tobaci* (Nimbalkar *et al.*, 1990), cotton boll worm, *Helicoverpa armigera* (Reddy and Rao, 1990). Neem cake/seed extract has been reported to be highly effective against *Spodoptera litura* to protect (Koshiya and Ghelani, 1990). Neem seed kernel has been found to be effective against pod borer of gram, *Heliothis armigera* (Butani and Mittal, 1990). Methanolic extract of deoiled cakê or 2% neem seed kernel suspension sprayed at 10 days interval was highly effective in protecting tobacco from caterpillar, *Spodoptera litura* (Murthy *et al.*, 1990). Neem derivatives have been found to control various pests of vegetables particularly the aphids and fruit borers (Mohan, 1989; Kathrival, 1988). Neem cake as the most effective material known today for nematode control has already been discussed.

Malaria control in India is at cross roads due to development of resistance among the mosquito species to DDT and HCH and increasing cost of these chemicals. Neem derivatives provide successful control of larvae. Neem oil has also been shown to break DDT resistance among the mosquitoes (Saxena *et al.*, 1989c). In spite of these merits, it has not been possible to popularize the use of neem products for malaria control. The reason is that malaria control is largely based on chemical control rather than on integrated multi-method approach and this is true in case of all other crop pests.

Registration of Neem-based Formulations

Till 1991, registration of neem-based products was not essential as these were considered safe and because the neem derivatives have been in extensive use in India for various purposes since time immemorial without any reported harmful effect. In 1991, the Central Insecticide Board decided to bring these under the Insecticide Act and has developed guidelines and data requirements for their registration for domestic use and export purpose (Table 1).

With the encouragement from all quarters, more than 2 dozens of neem products are being commercially sold and five of them have been registered (Table 2).

Future Research

The scenario presented here brings out 3 aspects of neem:

(a) Neem possesses a variety of chemicals which affect the survival of insects and nematodes through several different mechanisms; (b) the effectiveness of neem derivatives in pest control has been proved beyond doubt in large number of cases; and (c) neem products have been credited as ideal partners in integrated pest management.

Table 1— Data requirements for registration of 'Neem-based Products' under section 9 of the Indian Insecticides Act. 1968*

A. CHEMISTRY

(i) Name of the Plant(s) part to be used for extraction of the active ingredients/components; (ii) Method of experiment in detail clearly identifying the Chemicals as indicated at Sr. No. (iii). (iii)Formulated neem extract should contain not less than 1500 ppm of azadirachtin a.i. in "Kernal" based formulation and 300 ppm in "neem oil" based formulations. When the insecticidal a.i. is other than azadirachtin, its (their) name(s),quality & quantity to be indicated; (iv) Chemical identity of the ingredient of Sr. No. (iii) above; (v) Physico-chemical properties; (vi) Specifications of ingredient as indicated at Sr. No. (iii) above; (vii) Method of analysis of azadirachtin/other insecticidal a.i.(s), a.i. other than azadirachtin; (viii) Analytical test report; (ix) shelf life.

B. BIOEFFICACY

(i) Bioeffectiveness; (ii) Phytotoxicity; (iii) Compatibility with other chemicals; (iv) Purpose of manufacture; (v) Direction concerning dosage; (vi) Time of application; (vii) Waiting period; (vii) Application equipment; (ix) Information regarding registration status in other countries, if any.

C. TOXICITY

(i) Acute oral in rat and mice; (ii) Acute-dermal; (iii) Primary skin irritation, irritation to mucous membrane; (iv) Neuro-behavioural toxicity; (v) Reproductive toxicity; (vi) Carcinogenicity; (vii) Mutagenicity; (viii) Effect on spray operators (health records).

D. PACKAGING AND LABELLING

(i) Labels and leaflets as per IR 1971 existing norms; (ii) Type of packaging; (iii) Manner of packaging - container content compatibility; (iv) Specification for primary package; (v) Specification for secondary package; (vi) Specification for transport package; (vii) Manner of labelling; (vii) Instruction for storage and use; (ix) Information regarding disposal of used package; (x) Process of manufacturing indicating material, balance generation of wastes.

Source: Official Communication No.19-46/90-CIR II
dated : December 11, 1991.

*For registration of 'neem-based products' for export, the requirements at Sr. Nos. (viii) & (ix) under Chemistry and (iv) to (viii) under Toxicity are not prescribed. Under Bioefficacy only information on registration status of tender or trade enquiry from the importing country, and under packaging and labelling, only the packaging specifications of the importing country are prescribed.

Table 2— Commercially produced neem-based pesticidal products in India including those in the pipeline

Product	Active ingredient(s)	Activity claimed	Manufacturer
Amitul mosquito oil	Oil	Against mosquitoes	M/s Amitul Agrochem Pvt. Ltd., P.O. New Sheopuri Colony, Gorakhpur-273 001, U.P.
Godrej* Achook	Azdirachtin, Azadiradione, nimbocinol, epinimbocinol	Antifeedant repellent/deterrent, male sterility, molt/chitin inhibitor, change in sexual behaviour, growth regulator/disruptant, ovipositional deterrent, juvenile harmone, interferene in biosynthesis, toxicant, ovicidal oviposition deterrent, altered egg hatchability	M/s Bahar Agrochem & Feeds Pvt. Ltd. E-24, Late Parshuran, Bombay-415722 M/s Godrej Agrovet Ltd., Bombay-400074 (Mktd by)
Field Marshal	Azadirachtin	Antifeedant/repellent	M/s Khetiwadi Corner, Shiyabave, Vadodara-390 001, Gujarat
Jawan Crop Protector	Extracts containing azadirachtin	Antifeedant/repellent, disturbs growth and reproduction	M/s McDA Agro Pvt. Ltd. Bombay-400001
Margocide CK*	Azadirachtin and its 15 derivatives	Antifeedant, growth inhibitory, ovicidal, oviposition deterrent, nematicidal, chemosterilant	M/s Monofix Agroproducts Ltd., 52, 53, First Floor, Swimming Pool Complex, New Cotton Market, Hubli-580029
Margocide O K*	Azadirachtin (major ingredient)	Insecticidal	-do-

(Contd.)

Table 2-Contd...

Moskit	Oil	Mosquito repellent	M/s Investment and Commercial Enterprises, Bombay- 400 022.
Neemhit		Pesticidal	M/s Skylark Agro-chem, W 92 M.I.D.C., Phase II, Dombivali (East), Thane-421 204
Neemark	Azadiràchtin	Antifeedant/ repellent, nematicidal	M/s West Herbochem Pvt. Ltd., V.S. Marg, Bombay - 400 025.
Neemasol	Azadirachtin as major ingredient	Antifeedant/ repellent/growth disruptor	M/s E.I.D. Parry (India) Ltd., Dare House, P.B. No.12, Madras- 600 001
Neemgold	Azadirachtin, kernel extract	Antifeedant	M/s, Southern Petro Chemical Industries Corporation Ltd., 97, Mount Road, Madras- 600 032.
Neemguard	Extract concentrate	Repellent, metamorphosis disruption (safe to natural enemies of insects)	M/s Akshay Chemicals, Bombay 400 052 (Mktd by M/s Gharda Chemicals Pvt. Ltd., 48, Hill Road, Bombay-400 050)
Neemrich	Extracts	Neemrich I-Warehouse pests Neemrich II- Antifeedant	Technology by National Chemical Lab., Pune (snonymous with Margocide CK & OK)
Neemta 2100	Extract concentrate	Repellent, metamorphosis disruptor (safe to natural enemies of insects)	M/s A J. Chemicals (Mkted by M/s Kisan Brothers Pvt. Ltd., 311, New Cloth Market, Ahmedabad-380 002)

(Contd.)

Table 2-Contd...

Nethrin	Oil	Pesticidal	M/s Amitul Agrochem. Pvt. Ltd., P.O. New Sheopuri Colony, Gorakhpur-273 001, U.P.
Nimbecidine*	Azadirachtin	Antifeedant, repellent, metamorphosis disruptor, synergist	M/s T. Stanes and Co. Ltd., Coimbatore-641 018
Nimlin	Extract concentrate	Repellent, metamorphosis disruptor (safe to natural enemies of insects)	M/s Sunline Agro Chemicals, P.B.No.73, Sakri Road, Dhulia, Maharashtra State
RD-9* (Repelin)	Neem, *Pongamia*, *Annona* and castor products (azadirachtin 3000 ppm)	Antifeedant, repellent (safe to pollinators)	M/s ITC Limited ILTD Divn., Rajahmundry, Andhra Pradesh
Sukrina	Azadirachtin, meliantriol and other activities of *A. indica* & *Galedupa indica*	Antifeedant, repellent, reduces depression	M/s Conster Chemicals Pvt Ltd., Madras - 600 116
Swaticure		Pest repellent	M/s Swati Industries Pvt. Ltd., Moray House, Bandra, Bombay 400 050
Vapacide		Antifeedant	Technology by Regional Research Laboratory, Hyderabad
Wellgro	Neem kernel powder	Repellent, fungus inhibitory, antiviral, plant nutrition, N-loss prevention	M/s ITC Limited - ILTD Division, Rajahmundry Andhra Pradesh

*Registered with Central Insecticides Board

Inspite of its unlimited potential for pest control. there is lack of good neem products in the market for various purposes. This requires great deal of research efforts particularly on the following aspects:

(1) Fair evaluation of neem products for pest control under field conditions taking into account the final pest population, the crop yield, and the cost-benefit ratio. The neem derivatives could be used alone or as complementary/supplementary to chemical pesticides in order to reduce their use in the environment. These materials could also be tested with other methods of control.

(2) Development of suitable formulations of neem derivatives is vital for practical use of neem in pest control. The present day problems are photo, thermal and microbial degradation and deterioration of the neem formulations. Stabilization of the formulations with suitable adjuvants is a challenging task. Offensive smell in neem products is another problem in putting up acceptable formulations. Mosquito control requires special formulations which are cheap, ecologically safe and free from offensive odours for use in water bodies, tanks etc.

(3) 'Neem oil extractive' is a by product of neem oil refineries and is presently a waste product. This should be converted into suitable formulation to open a remunerative outlet for it.

(4) Neem derivatives possess anti-microbial property. However, this has not yet been widely explored to control bacterial and fungal diseases particularly on horticultural crops.

(5) Neem is a repository of so many chemicals, several of them still identified. Research needs to be strengthened on identification and evaluation of their efficacy in pest and disease control.

(6) Neem derivatives can be used to produce pesticide granules with controlled release either alone or in combination with synthetic chemicals.

(7) Neem cake is excellent nematicide. But its stable formulations are lacking particularly for use in those places/countries where neem is not grown.

(8) Genetic diversity need to be explored for higher yield of bioactive material and higher oil content.

While concluding, it must not be forgotten that there is vast potential for export of neem derivatives in suitable formulations. In fact, neem is gold mine for those who wish to invest in pest control materials.

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Advances in Neem Research and Development "Present and Future Scenario"

Opender Koul

Regional Research Laboratory
Canal Road, Jammu 180 001
India

IN recent years the broad spectrum Chemical pesticides which are toxic towards variety of insect species, have been shown to indiscriminately destroy other beneficial species including the natural enemies of various pests. Resistance towards these chemicals is an additional factor which involves increase in the amounts of pesticide usage thereby causing increased ecological disturbance and pollution of the environment.

The need for environmentally safe plant protection methods has stimulated the search for new class of insecticides and accordingly plant chemical defences against insect attack have been chosen as one of the means in present day IPM system. Such chemicals are useful because their mode of action is/could be in direct contrast to more traditional pesticides and insecticides. Today Indian neem tree, *Azadirachta indica* A.Juss, is a classical example from Meliaceae family due to the unique characteristics of its, limonoids and other secondary metabolites, and which has potential use in pharmaceutical, manufacture of agriculture implements, cattle and poultry feeds, nitrification of soil and pest control.

By 1991 the scientific literature dealing specifically with neem and its products has reached approximately 1400 articles and accordingly substantial literature documents the description of this tree including distribution, propagation and growth (Koul *et al.*, 1990, Randhawa and Parmar, 1993) which has been presented

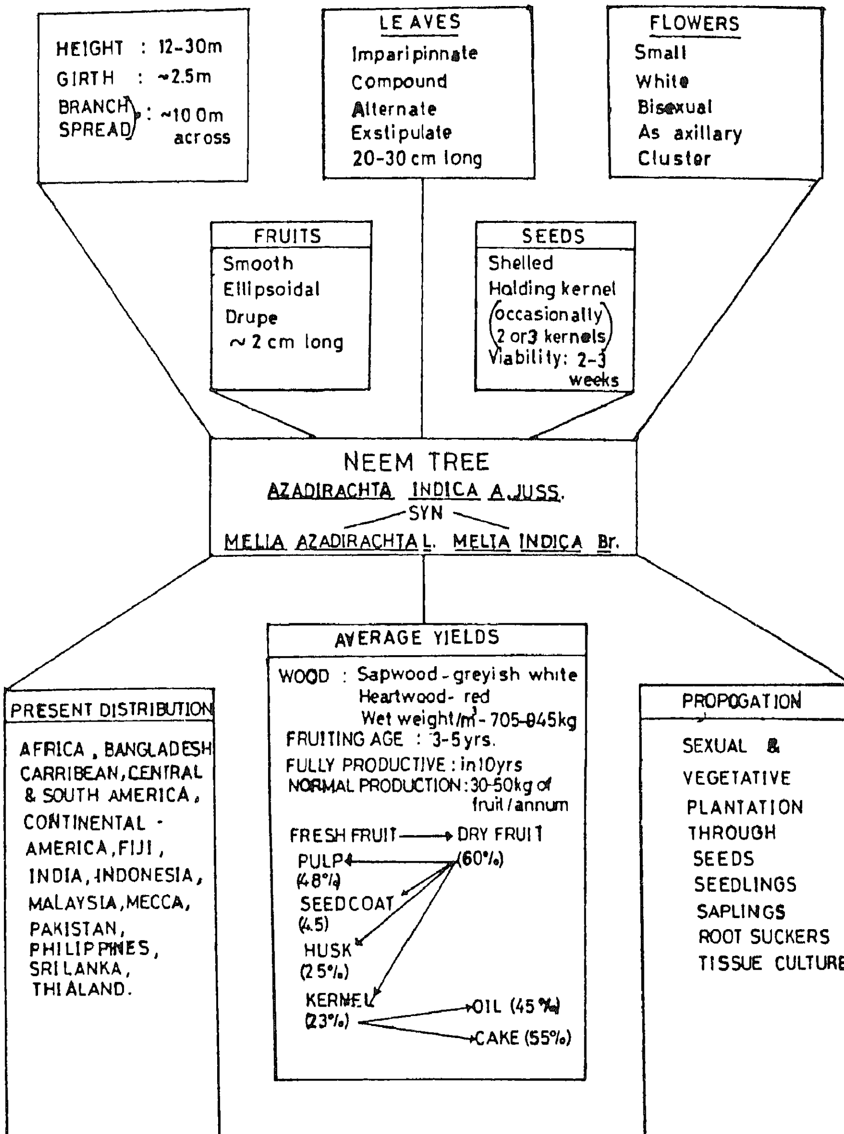

HEIGHT : 12-30m
GIRTH : ~2.5m
BRANCH SPREAD : ~10 0m across
LEAVES
Imparipinnate
Compound
Alternate
Exstipulate
20-30 cm long
FLOWERS
Small
White
Bisexual
As axillary
Cluster
FRUITS
Smooth
Ellipsoidal
Drupe
~ 2 cm long
SEEDS
Shelled
Holding kernel
(occasionally 2 or 3 kernels)
Viability: 2-3 weeks
NEEM TREE
AZADIRACHTA INDICA A.JUSS.
SYN
MELIA AZADIRACHTA L. MELIA INDICA Br.
PRESENT DISTRIBUTION
AFRICA, BANGLADESH
CARRIBEAN, CENTRAL & SOUTH AMERICA,
CONTINENTAL - AMERICA, FIJI,
INDIA, INDONESIA,
MALAYSIA, MECCA,
PAKISTAN,
PHILIPPINES,
SRI LANKA,
THIALAND.
AVERAGE YIELDS
WOOD : Sapwood - greyish white
Heartwood - red
Wet weight/m³ - 705-845kg
FRUITING AGE : 3-5yrs.
FULLY PRODUCTIVE : in 10yrs
NORMAL PRODUCTION : 30-50kg of fruit/annum
FRESH FRUIT
DRY FRUIT
(60%)
PULP
(48%)
SEEDCOAT
(4.5)
HUSK
(25%)
KERNEL
(23%)
OIL (45%)
CAKE (55%)
PROPOGATION
SEXUAL &
VEGETATIVE
PLANTATION
THROUGH
SEEDS
SEEDLINGS
SAPLINGS
ROOT SUCKERS
TISSUE CULTURE

Fig. 1

Neem trees have been extensively cultivated in the old world tropics. In the new world tropics, some neem trees are almost century old, but importance of this species in these regions has been ignored until recently. Today this tree is becoming the first choice of propagation in the Caribbean islands, Central America and South America (Pliske, 1984) and is even being propagated in the continental United States.

At the U.S Department of agriculture station in Mayaguez, Puerto- Rico, neem trees are now fourteen years old. These trees were grown from greenhouse transplants when they were close to 1m in height. (Jocabson, 1987). Direct seeded plants establish a good root system before aerial growth becomes rapid (National Academy of Sciences U.S.A, 1980) and can substantially reduce the cost of transplantation. In Arizona, the goal of developing a neem tree with frost resistance to -8°C, using seeds from northern India, is under investigation (Jacobson 1987). In the Philippines, a new programme has been established for crop production and harvest protection of neem (Baluyut 1984).

Neem seedlings are usually propagated in the nursery and transplanted to the field, although direct sowing has been successful under conditions of adequate rainfall. In the nursery, the seedlings are grown in plastic bags, root trainers, or seed beds. Germination begins in about 7-8 days and continues up to 3-5 weeks. Transplantation of seedlings is recommended when they reach a height of 15-20 cm. However, Troup (1921) suggested transplantation when the height reaches 0.8-1.0 m, whereas Radwanski (1977a) suggested 7.5-10.00 cm. Transplantation of stumps is well-known in India; these are prepared from 2-year-old seedlings and are subsequently planted in 30 cm^3 pits after pruning (Singh 1982). Root-ball transplantation is another good method where 1-year-old seedlings are carefully uprooted along with a ball of soil around the roots and transplanted as soon as possible. Spacing between plants is considered to be an important factor, and in Nigeria a spacing of 1.8 × 1.8 m is recommended (De Jussieu 1963).

To propagate the neem tree widely as a commercial crop, germination characteristics have to be better understood. Some initiatives have already been taken in Mauritius (Fagoonee 1984), and enhanced germination may be possible in the future. However,a more effective procedure could involve propagules obtained via tissue culture techniques. Little effort has been made in this direction, but some encouraging reports, such as the differentiation of growth centres in *A. indica* callus (Rangaswamy and Pomilla, 1972); root formation on Murashige and Skoog medium containing IAA (Sanyal *et al.,* 1981), and callus formation in some African progeny (Schulz, 1984). Similarly induction of somatic embryogenesis from immature cotyledonary tissues of *A. indica* has been determined (Shirkhande *et al.,* 1993) which may provide a basis for future research. Propagation of neem by cuttings is also promising, requiring a production period of 6-12 months.

In summary, neem is becoming a highly preferred tree for propagation, not only within its native range, but particularly in the Western hemisphere.

Neem Chemistry

Advances in the Chemistry of the neem have been rapid as a result of interest in the bioactivities of this tree. Although recent reviews have been published (Rembold, 1989; Koul *et al.*, 1990; Schmutterer,1990. Lee *et al.*, 1991; Dev Kumar and Sukh Dev 1993), more precise information is rapidly being generated.

Chemical investigations of the neem tree, *A. indica* have yielded many characteristic compounds with interesting structures. Tetranortriterpenoids (Limonoids) are most characteristic and major class of secondary metabolites and major emphasis of discussion will be on these compounds.

In recent years number of tricyclic diterpenes have been found having an aromatic C-ring with oxygen functionalities at C-3 and C-7 (Hausen *et al.*, 1994) (Fig. 2).

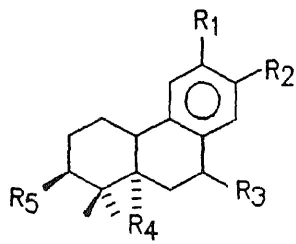
	R_1	R_2	R_3	R_4	R_5
1. Methyl nimbiol	OCH_3	CH_3	=O	H	H_2
2. Methyl nimbionone	OH	i-pr	=O	H	H_2
3. Nimbidiol	OH	OH	=O	H	H_2
4. Nimbonone	C_2H_5	OCH_3	=O	H	H_2
5. Nimbonolone	C_2H_5	OCH_3	H_2	H	=O
6. Nimbosone	OCH_3	OCH_3	H_2	H	H_2
7. Nimolinin	OH	i-pr	=O	H	=O
8. Nimosone	OH	i-pr	=O	OH	H_2
9. Sugiol	OH	CH_3	=O	H	H_2

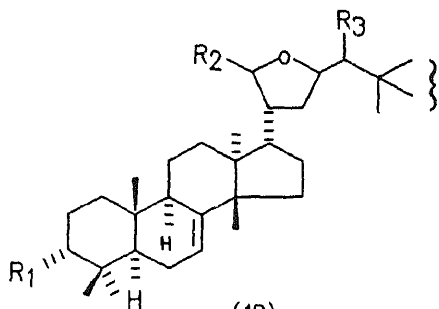
Fig 2. Tricyclic diterpenes of neem

Nimbonone (4) and nimbonolone (5) have been recently isolated from the neutral fraction of the stem bark of *A.indica* (Ara *et al.*, 1989a) having a unique feature in C-12 ethyl group. Similarly from stem bark of this tree, the compounds like nimosone (8), nimbosone (6), methyl nimbiol (1) and methyl nimbionone (2) have been isolated as new compounds (Ara *et al.*, 1988). Two new tricyclic diterpenes nimolinin (7) and nimbidiol (3) have been isolated from root bark (Ara *et al.*, 1989b; Majumder *et al.*, 1987).

So far seven tetracyclic triterpenes (10) (protomeliacins) have been isolated from various parts of neem tree (Hansen *et al.*, 1994) which include meliantriol, nimbocinone, nimolinone, azadirachtol, azadirachnol, azadirol (from neem fruits) and kulactone.

As mentioned above the major class of compounds in neem is of meliacins (limonoids) which are triterpene derivatives in which four side chain carbon atoms have been lost and their remaining carbon atoms have been cyclized into a furan





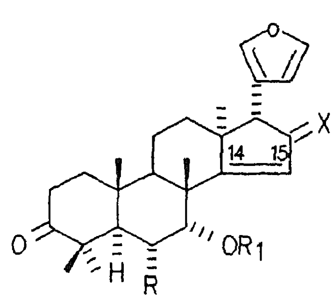
(10)

ring. One or more of their ring systems may have also undergone oxidative opening. All the known meliacins possess an oxygen at C-7, a double bond in ring-D and a methyl group at C-8. These can be differentiated into various categories like:

(i) limonoids with intact ring system

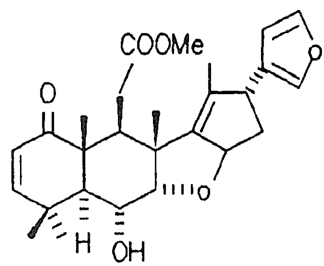
(ii) limonoids with open ring system

Intact ringed limonoids (11) are related to tetracyclic triterpenoids such as azadirone, azadiradione, epoxyazadiradione, azadiradione-7-benzoate, meldenin, vilasinin, and vilasinin triacetate which have an intact D-ring, but have been oxidized to include an epoxide ring at C-14 and C-15.



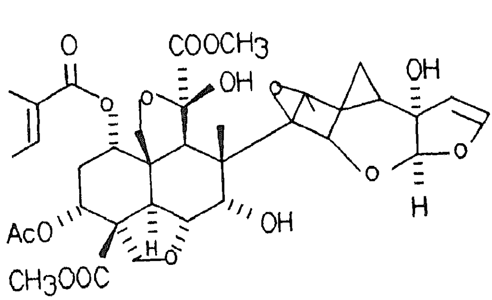
(11)

Ring opened limonoids (12) include those compounds in which one or more of the rings has been opened or altered by oxidation. Mostly C-ring opened compounds are common in *A. indica*, such as nimbin, salannin, nimbandiol, nimbinene, desacetyl-nimbinolide and so on. The only A-ring opened compound is 4a, 6a -dihydroxy-A-homoazadiradione (Bruhn *et al.*, 1984). Similarly D- ring opened compound nimolicinol is one of the examples of this group (Siddiqui *et al.*, 1984).



(12)

The most highly oxidized limonoids in neem are those where both C and D rings are modified. Azadirachtin (13) is the most studied molecule, predominantly found in neem seed kernel occurring typically in levels of 0.2 - 0.3% on dry kernel weight basis. However, in certain ideal natural conditions 0.6-0.7% of azadirachtin has also been reported (Hansen *et al.*, 1994).



(13)

Today number of azadirachtin derivatives are known to occur in neem (Ley *et al.*, 1993). They are azadirachtin A to Azadirachtin K. Out of these azadirachtin A (13) or commonly called azadirachtin is the predominant bioactive compound. Only a partial structure comprising a trans-decalin substituted as for azadirachtin-A is available for azadirachtin-C. Azadirachtin-D differs from azadirachtin-A in that C-29 is not oxidized and is present as an angular CH_3-group. Similarly azadirachtin E, F and G have structural relations of 3-tigloyl-azadirachtin (Rembold, 1988). In

case of azadirachtin-F the unoxidized C-19 angular methyl group is accompanied by a pendant methyl glycolate side chain at C-9, while for azadirachtin-G C-13 and C-14 oxirane is absent and replaced by double bond and a C-17 hydroxyl group (Koul, 1992).

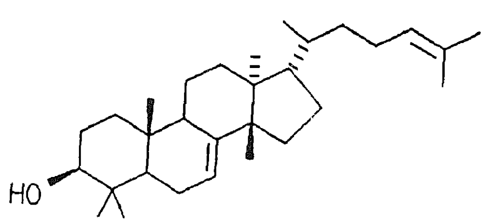
Azadirachtin H and I have recently been isolated from neem seed kernel by preparative HPLC (Govindachari *et al.*, 1992a) and found to lack C-12 carbomethoxy group. Similarly recently discovered azadirachtin-K contains a β-keto-δ-lactone between C-19 and C-12 in place of the five-membered lactol ring (Govindachari *et al.*, 1992b). Recently the compounds like vepaol and isovepaol, which are dihydro 23 α- and β-methoxy azadirachtins, have also been claimed to be naturally occurring compounds in neem (Kraus *et al.*, 1985; Sankaram *et al.*, 1987).

Full structural determination of azadirachtin was first proposed in 1975 by Nakanishi and his coworkers. However, simultaneous publications of Kraus *et al.*, (1985) and Bilton *et al.*, (1985,1987) reported the correct structure of azadirachtin using NOE differential spectroscopy and single crystal X-ray analysis respectively.

In recent years substantial synthetic approach, particularly for the synthesis of azadirachtin has been taken up by Ley and his coworkers (Ley *et al.*, 1993) and Shibasaki and co-workers (Nishikimi *et al.*, 1989). There has been a success in synthesizing decalin and furopyran units. These groups plan to transfer these advanced synthetic intermediates into azadirachtin using aldol-like methodology following hydroxylation of furano-unit at the pro-C-13 carbon atom (Ley *et al.*, 1993). However, despite the massive effort, over nearly a decade, no synthesis is near completion.

Biogenesis

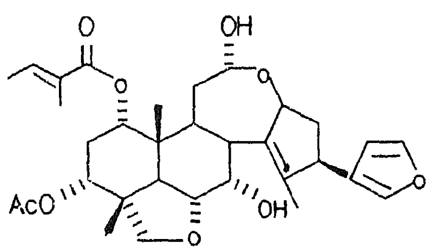
For the elaboration of azadirachtin, the biosynthetic pathway in neem is unknown. However, various schemes have been proposed for the biogenesis of tetranortriterpenoids including azadirachtin (Connolly, 1983; Champagne *et al.*, 1992). According to the current view the biogenesis is supposed to begin from tetracyclic triterpenes Tirucallol (14) or its, C-20 epimer euphol and has been comprehensively described in a recent review on limonoids in rutales (Champagne *et al.*, 1992).



(14)

The isolation of several intact tetracyclic triterpenes from various parts of neem tree (e.g.: azadirachtol, meliantriol, nimbocenone, nimolinone etc.) in which the oxidation of C-17 side chain has occurred; would suggest that partial formation of the limonoid furan ring precedes the rearrangement of the carbon skeleton. Following formation of the basic limonoid skeleton, a variety of oxidations and skeletal rearrangements may occur (Champagne *et al.,* 1992). Commonly the D-ring is oxidized to a lactone. In the series azadiron, azadiradion, 14-epoxyazadiradion and gedunin introduction of a carbonyl function at C-16 followed by Baeyer Villager oxidation yields a lactone.

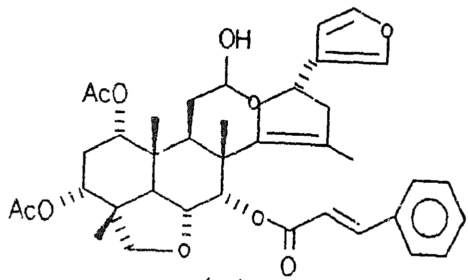
There is controversy about the mechanism by which the C-12/C-13 bond of the C-ring is opened. Mitra *et al.,* (1970) and Siddiqui *et al.,* (1988) suggest that the C-12/C-13 bond is cleaved to form a 12-acylium ion followed by attack of the OH-7 on C-15. Taylor (1984) proposed that cleavage of the 12-13 bond is accompanied by the simultaneous opening of a 14,15-epoxide to generate CHO-12 and OH-15 functions; rotation about the 8-14 bond would allow the OH-15 to recyclize with the CHO-12 to form a lactol C-ring as in Volkensin (15). This latter mechanism has been supported by Rajab *et al.,* (1988) who isolated both salannin and volkensin from *Melia volkensii.*



(15)

Ring opening of the hemiacetal of nimbolin B (16), followed by rotation about the 8,14 bond and nucleophilic displacement by the 7-α-OH on the protonated 15-OH, coupled with transesterification of the C-1 acetate to tiglate and oxidation of C-12 to methyl ester would afford Salannin. Similarly a scheme has been proposed for the conversion of salannin to azadirachtin by rearranging ring-D, epoxidation of C-20/C-21 bond of the furan ring followed by oxirane ring opening to afford a trans diol (Hansen *et al.,* 1994).

Most of the biogenetic proposals however, are tentative as they are not supported by valid biosynthetic studies. There is only one instance of biosynthetic investigation in neem, that of nimbolide in neem leaves (Ekong *et al.,* 1971; Ekong and Ibiyami, 1985). These studies show that euphol is more efficiently incorporated



(16)

into nimbolide as compared to other suggested precursors, thereby suggesting that the biosynthesis of C-secomeliacins may involve the $^{7,9(11)}$ diene which can easily be formed from an 8α, 9α-epoxide. These studies also suggest that the biogenetic pathway for C-secomeliacins as different from others.

Similarly biogenesis of nimbinene can be anticipated by C-28 decarboxylation on nimbin, desacetylnimbin etc. In this case apparently C-28 oxidation and decarboxylation precedes those of C-29 in the biogenetic pathway because of the occurrence of several nimbin analogues containing C-28 oxygen function (Dreyer, 1984). In fact nothing is known about the cellular location of enzymology of limonoid biosynthesis and all pathways of biosynthesis are characterized by evolutionary patterns of increasing oxidation and rearrangement of the original limonoid skeleton (Champagne *et al.*, 1992).

Aflatoxins

Aflatoxins are potent carcinogens/toxins which can be a cause of major concern at the time of neem seed processing for bioefficacious preparations. Fungal infection of the moist seed is a result of the conditions at harvesting time. This is due to poor handling of moist seed, which creates the conditions for the development of fungus, *Aspergillus flavus* in particular, and ultimately the substantial production of aflatoxins. This problem, however, can be minimized by improving collection practices, handling and storage. The key factors for this are speedy depulping of fruit, keeping moisture content of seeds around 10%, and storage of dried seed away from sun and rain.

Recent advances in the processing of seed extracts have helped in minimizing aflatoxin contamination and it is now possible to efficiently remove aflatoxins to levels below that of public health concern.

In recent years the studies in USDA concerning neem's effects on certain fungi have shown that aflatoxin production are reduced, though neem leaves the microbe alive, but apparently switched off the aflatoxin biosynthesis (Bhatnagar, *et al.*, 1990). These results are promising and if the components responsible for this type

of bioactivity are formed in neem, it will be easy to control aflatoxin synthesis and inexpensive method for protecting stored foods from aflatoxins. This is especially significant these days, as with the availability of even more sensitive chemical analyses, health officials are becoming alarmed at aflatoxin's widespread occurrence and potential hazard.

Greenhouse studies have since confirmed that neem extracts appear to halt the formation of a substance called polyketides, which the fungi convert into aflatoxin. The enzymes for the conversion remain in place, but key chemicals they need to synthesize the feared toxin are no longer available.

Bioefficacy

As Anti-insect

The performance of neem products as insect control agents has been assessed in terms of antifeedant and insect growth regulatory (IGR) effects. The cornerstone scientific report regarding the antifeedant action of neem against insects originated from the Indian Agricultural Research Institute in 1960 and was subseqently confirmed under field conditions (Pradhan *et al.*, 1962). Today numerous studies are available describing the insecticidal, antifeedant, growth inhibitory oviposition deterring, antihormonal and antifertility activities of neem against broad spectrum of insects (Mordue and Blackwell, 1993). These biological activities have been demonstrated for the oil expelled from seeds and leaves, leaf extracts, seed extracts, neem cake, fruit extracts and various isolated compounds (Koul, 1984a,b; Koul *et al.*, 1990; Koul, 1992).

For insect control these materials have been evaluated against a wide range of pest insects on vegetables (Cabbage, cauliflower, Chinese kale, crucifers, cucurbits, beans, egg plant, onions, solanum and potato); ornamental crops, stored grains and also against house hold pests of medical and hygienic importance. A number of comprehensive reviews and proceedings in this regard have been published (Warthen, 1979; Jotwani and Srivastava, 1981 a,b; Jacobson, 1986; Koul, 1992, Koul and Smirle; 1993 Saxena, 1989, Schmutterer, 1988; Schmutterer and Ascher, 1984, 1987; Randhawa and Parmar, 1993;).

Of all the isolated active compounds from neem only azadirachtin stands out in terms of its bioefficacy and large amount of data detailing both feeding deterrency and the growth disruptive properties. In recent years cotton crops have received particular attention (Phadke *et al.*, 1988; Flint and Parkes, 1989) together with corn (Klocke and Baruby, 1989), Potato (Kaethner, 1992) and more diverse crops such as chrysanthermums (Knodel *et al.*, 1986) and Mahogany (Howard, 1990). Laboratory trials have been extended to the field (Kaethner, 1992) with azadirachtin and neem extracts often showing speriorty to certain commercially available pesticides (Kirsal, and Schmutterer, 1988; Isman *et al.*, 1990, 1991).

Some insect species resistant to conventional pesticides have also shown to get effected by neem products (Hummel, 1989).

Azadirachtin has been shown to have a potential in the aquatic environment as well, where mosquito larvae have been effectively controlled (Zebitz, 1987; Koul,1988; Al-Sharook *et al.,* 1991). The mode of action of Azadirachtin is still not entirely clear. So far the studies show that it is antihormonal and anti-behavioural in a broader sense. The differences in azadirachtin effects between insect species are apparently due to more than one site-of-action and depends on time, mode and stage of treatment (Koul *et al.,* 1987, 1994, Mordue and Blackwell, 1993).

Other active isolates from neem have been mostly evaluated as antifeedants only and about 25 such limonoids deter feeding in insects. Recently two new compounds isolated from winter neem leaves, isonimocinolide and nimocinolide, have been shown to have growth regulatory effects, resulting in abnormal larval and pupal development (Naqvi *et al.,* 1991).

As Nematicide

The neem cake has been the most favourite neem derivative tested against plant-parasitic nematodes attacking a wide variety of crop plants. It has been used as soil amendment against variety of nematodes in tomato, egg plant, Chilie, okra, black gram, chick pea, green gram, French bean, Papaya, betelvine, tobacco, cabbage, cauliflower, grape vine, plum, sugarcane and berseem (Alam, 1993). There are three basic principles involved in such control of nematodes; habitat management,host modification, and direct toxicity of the neem products, which are released through root-exudation and decomposition of plant residues.

Use of neem as a means of biological control of nematodes is also well-documented. The planting of neem saplings around highly susceptible varieties (e.g. tomato, egg plant, cabbage, cauliflower) in nematode-infested soil reduces the nematode population (Alam, 1990).

Root dipping and seed treatment with azadirachtin prevents larval *Meloidogyne incognita* from causing root-knot infections (Siddiqui and Mashkoor, 1988; Abid and Maqbool, 1991; Pradhan *et al.,* 1991). Interestingly, the residual effect of soil treatments for nematicidal control lasts for a number of months. Even in the country like India where soil conditions can vary drastically between areas, neem efficacy was consistent all year round, in all soil conditions (Alam, 1991).

The present state of knowledge of nematicidal nature of neem should serve the basis for developing such neem based formulations which could be applied with the conventional methods so that such products would be more acceptable to farmers. Several reports indicate the utilization of neem derivatives in IPM system particularly with biological control of nematodes and such researches need to be further strengthened.

As Fungicide

There are several instances of the effects of neem preparations or azadirachtin on fungal pathogens. Antifungal activity of neem preparations against soil borne pathogens of chick pea (Singh *et al.*, 1986; fruit rot disease of cucurbitaceous plants (Krishna *et al.*, 1986; Sinha and Saxena, 1986; spore germination and mycelial growth on finger millet (Jagannathan and Narasimhan, 1988; fungus of Paddy (Shetty *et al.*, 1989; rust disease of ground nut (Muthusamy *et al.*, 1988; foot-rot pathogen of barley (Singh and Dwivedi, 1990) is fully established. An exhaustive study on the utility of neem oil against fungi has been carried out by various workers. Jain and Agrawal (1978) has shown effects of neem oil against some keratinophilic fungi. A significant control by neem oil of *Fusarium, Aspergillus, Drechslera*, and *Macrophomia* (Vir and Sharma, 1985 a,b) has been recorded at concentrations of 25-50%. Jeyarajan *et al.* (1987) used neem oil and other plant products in the management of plant diseases in India and addition of neem cake to soil reduced the pre-emergence and post-emergence mortality of cotton seedlings infected with *Rhizoctonia solani*. Certain fruit rots are known to cause huge economic losses and their control by neem extracts in the laboratory have shown considerable promise (Arya, 1988), thereby advocating the development of field trials in affected areas.

However, the present scenario in terms of antifungal effects; neem is not universally effective against fungal pathogens. Khan *et al.*, (1988) could show no effects of neem on 14 common pathogenic fungi which were dermatophytes, yeasts and moulds. Apparently the neem bioefficacy as antifungal is still of preliminary nature and, therefore, it is highly desirable to further explore and then exploit the possibilities for practical application and control of pathogenic fungi.

As Antiviral

There are several reports on the use of neem and its products against viruses. Green-house trials against tobacco mosaic virus (Mishra and Rao, 1988) and cowpea mosaic virus (Singh *et al.*, 1988) have been successful with the help of neem products. Similarly the isolates from neem like nimbidin and nimbin (from neem oil) were active against potato virus (Verma, 1974) at 1000 ppm. However, neem oil itself was highly effective against transmission of Grassy stunt virus and Ragged stunt virus disease of rice (Saxena and Khan, 1984). Rice tungro virus is also controlled by neem seed Kernel powder plus carbofuran (Kareem *et al.*, 1988). Leaf and bark extracts of neem inhibited infection of *Chenopodium amaranticolor* by cowpea mosaic comovirus (Singh *et al.*, 1988) and Spinach mosaic virus (Zaidi *et al.*, 1988).

It, however should be noted that practically nothing has been done on the large scale use of neem products as antiviral agents, though lab scale experiments have shown such protective effects. For soil-borne viruses and even fungi crude deriva-

tives of neem might prove highly promising and attempts should be made to evaluate such products on large scale in the field situations.

It should also be noted that practically nothing has been done on the use of neem against plant pathogenic bacteria. Therefore, thorough investigations to check bio-activity of neem against plant-pathogenic bacteria need concerted efforts.

Medicinals

It is apparent that different parts of neem were used in ancient Ayurvedic veterinary preparations to cure various animal diseases (Singh, 1993). It is also observed that neem has been used almost invariably as one of the components of the multi-ingredient recipes used as remedy. There are several references in Vedas which point to medicinal value of this tree and mention in Agni Purana, Bhava Prakash, Charak Samhita, Dravya Guna Vijnana, Sushruta Samhita, Vangasen Samhita and Astang Hridayam (Pandey, 1993) possess substantial evidence for this.

All parts of neem tree viz. roots, stem, leaves, flowers, fruits, seeds, bark and gum have therapeutic uses. The pharmacological action and the therapeutic efficacy of neem, in general, as reported in Unani texts includes anti-inflammatory, concoctive, blood purifying, anti-leprosy, anti-vitiligo, antiflatulent, wound healing, antiseptic, anti-arthritic, anti-prulent, anti-pyretic, anti-microbial and anti-helminthic actions (Rahman and Jairajpuri, 1993).

Present knowledge regarding neem oil and its isolates nimbidiol, nimbidin, and diethyl sulfides show their efficacy in controlling mycobacteria and various pathogens (Koul *et al.,* 1990). Certain organosulfur compounds have been found to be potent antileprotic agents. These sulfones (Gupta and Joshi, 1983) along with aqueous leaf extracts also act as anti-malarial substances (Obaseki and Jegede-Fadunsin, 1986). Nimbolide extracted from the dried leaves inhibits *Plasmodium falciparum*. Nimbinin also shows anti-malarial activity (Bray *et al.,* 1985). Neem has schizontocidal activity but the action is not sufficiently pronounced as compared to the currently available drugs eg. Chloroquine, quinine, artemisin etc. Recently a most active anti-malarial constituent from *A. indica* has been identified as gedunin (Khalid *et al.,* 1989). Obviously the question arises whether there could be any scope for neem in the treatment of malaria. It is too early to come to any conclusion, particularly when the studies are scanty and activity has mostly been examined as schizontocidal drug but not as casual prophylaxis anti-relapse, sporontocidal and gametocytocidal. Therefore, there is a scope for further exploration on the action of neem or its components on other stages of malarial parasite, including its potentiating action.

Dermatological effects of neem products are significant as they provide relief from various skin diseases without side effects. Neem oil and some pure isolates (nimbidin, nimbidiol and nimbin) can also inhibit fungal growth on animals and humans (Koul *et al.,* 1990). Extracts of neem plant totally inhibited the protease activity of *Trichophyton* sps. (Iyer and Williamson, 1991). Jacobson (1986) has

also reported high antimycotic activity in neem preparations. Some anecdotal information attributing to antiviral activity of neem is also available particularly against pox-viruses using paste of neem leaves. A 10% aqueous extract of leaves prevented viral skin infection in rabbits and monkeys (Rao *et al.*, 1969). Leaf extracts also cure acute eczema and Scabies (Singh *et al.*, 1979). Sankaram (1987) and his coworkers have recently isolated a fraction-G from fresh neem Kernels that exhibited antiviral activity against the Ranikhet disease virus of poultry at 1mg/embryo both *in vitro* and *in vivo* studies.

Recent pharmacological studies have also supported the belief that neem leaves possess some anti-viral activity; though results are unconfirmed. In USA aqueous neem leaf extracts have shown low to moderate inhibition of the viral DNA polymerase of hepatitis-B virus (NRC, USA; 1992). In Germany, an ethanolic neem-kernel extract has proved effective against herpes virus. In horticulture studies, crude extracts also seemed to effectively bind certain plant viruses, and thus limit infection (NRC, USA, 1992).

Analgesic and antipyretic effects of neem components are also encouraging, though most of the studies are related to animal tests. Some clinical trials with eosinophilic patients have shown that a 100 mg dose three times daily for 10 days reduces eosinophil count by 75% (Koul *et al.*, 1990). Among major isolates only nimbin, nimbidin or sodium nimbidate have been shown to exhibit antipyretic effect in guinea pigs, with anti-inflammatory and anti-histaminic properties as well (David, 1969, 1978; Pillai & Santhakumari 1981). However, most of these studies are carried out by systemic administration and need definitive model system development to establish the traditional claims and to develop a standard drug.

Epidermoid carcinoma and parotid tumours were successfully treated with neem seed oil injections in an Indian patient (Chaterjee, 1961), but no conclusions should be drawn from this sole report. Some types of cancer in mice undergo remission when treated with neem preparations (Hartwell, 1983). *In vitro* studies with neem limonoids, 7-acetylneotrichilenone and 1,2-diepoxy-azadiradione against the murine P-366 lymphocytic leukemia cell line showed that only the former was effective with an ED_{50} of 8.5μg/ml (Pettit *et al.*, 1983).

Another widely studied medicinal property of neem has been its antifertility action. In some earlier studies sodium nimbinate and sodium nimbidinate have shown spermicidal activity in rats (Sharma and Saksena, 1959 a,b). In more recent studies single or multiple intra-vaginal application of neem oil during pre- or post-implantation period prevented pregnancy in rats (Sinha *et al.*, 1984) and has further been confirmed in rhesus monkeys (Bardhan, *et al.*, 1991). Upadhyay and his coworkers (1990) have observed that in rats, injection of neem oil (Single dose of 100 μl) into the uterine horn, created an immunological response and prevented pregnancy for nearly five months. This is an interesting finding and needs extensive investigation. The overall information on neem as a contraceptive is briefly summarized in Fig. 3.

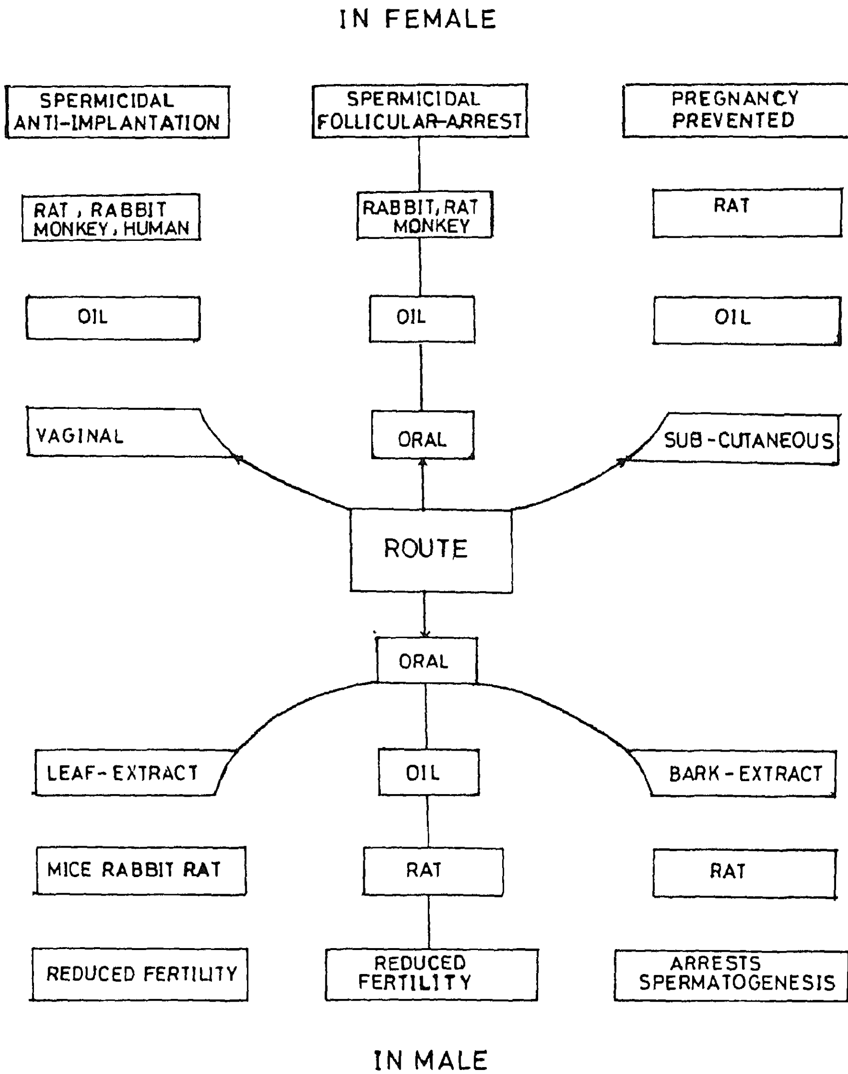


Fig.3. Neem as Contraceptive.

In a recent development a single administration of purified neem extract "Praneem" into the uterus causes a long lasting effect on fertility in rats and monkeys without any disturbance of ovulation and sex steroid hormone production (Talwar *et al.*, 1993). A polyherbal cream developed by this group employing "Praneem" and quinine hydrochloride for intravaginal use has high contraception efficacy in rabbits and monkeys. This preparation has been approved for clinical trials in patients with leukorrhea and infections with human papilloma virus (Talwar *et al.*, 1993).

Neem also acts as antihyperglycemic agent. The oil of seeds does not produce hypoglycemia in normal animals (Bhargava, 1989), but it does improve glucose tolerance in partially pancreactemised dogs. It has been reported that within 5 hours blood sugar levels could be reduced by approx 25% on an average in rabbits (Pillai and Samthakumari, 1981). Clinical trials on non-insulin dependent human volunteers indicate that such activity is a synergistic one with most of the oral antidiabetics and thus help in gradual reduction of the allopathic drug intake (Riar, 1993).

Another important pharmacological use of neem material is as a dentifrice reputedly producing remarkable healing of gum inflammations and paradontosia. Stomatitis is also known to be cured by an extract from bark of the neem tree (Lorenz, 1976).

On the whole medicinal studies of neem preparations have been done and a wide variety of activities recorded. In many cases the results are promising but in most of the studies the follow-up is limited and in only few cases active constituents have been identified. Though chemically a number of pure compounds are known (Nigam *et al.*, 1988), pharmacological evaluation of these compounds need to be undertaken to explore their practical potential.

Toxicology

On the whole data on the toxicology of various neem preparations is scanty. Some experimental studies have demonstrated toxic effects of few isolated compounds, neem oil, extracts or from different parts of the tree against mammals or non-mammalian vertebrates. Gastrointestinal spasm, lethargy and hypothermia in mice was induced by 75% methanolic extract of neem leaves at 13μg/kg dose (Sinnials *et al.*, 1983; Okpanyi and Ezeukwu, 1981). Ali (1987) reported body weight loss and diarrhoea in guinea pigs which were orally fed with 50-200 mg/kg aqueous suspension of green or dried neem leaves. Such extracts also caused heptobiliary disease in albino rats by raising 5-nucleotidase to an abnormally high level (Obaseki *et al.*, 1985). The oral and dermal administration of neem seed cake to rats caused no carcinogenicity in chronic studies (Sardeshpande, 1976). However, water extract of neem seed cake has potentiation effect on the sleeping time induced by pentobarbitone sodium by interfering in hepatic microsomal enzyme system through which the latter is metabolised (Vijjan and Tandon, 1985).

Bhide and his coworkers studied toxicology of sodium nimbidinate, an isolate from neem as early as 1958a in mice (acute LD_{50} = 700 mg/kg, intraperitoneal) and rats (acute oral LD_{50} = 1000 mg/kg, oral). Histological changes observed in liver and renal tubules show proliferation of endothelial cells in the glomerular apparatus, and the renal tubules exhibit cloudy swelling, fatty infiltrations and necrosis in convoluted segments. Sinniah and Baskaran (1981), while reporting about liver biopsey of one human infant and necropsy examination of ICR strain mice after neem oil poisoning have observed pronounced fatty infiltrations of the liver and mitochondrial damage in proximal kidney tubules. However, albino rats and mice were unaffected when fed nimbidin up to 2000 mg/kg or when administered intraperitoneally at 1000 mg/kg. Dogs fed nimbidin at 10 and 20 mg/kg for 4 weeks showed no systemic toxicity (Pillai and Santhakumari, 1984).

Some toxicological observations from human subjects when given neem seed oil as an antihelmintic include occasional nausea and general discomfort (Chopra *et al.*, 1965). However, sodium nimbidinate fed equivalent to 7g/person or injected equivalent to 1g intramuscular elicited no local or general side effects (Bhide *et al*, 1985b). Involvement of neem oil in the etiology of Reye's syndrome has been indicated and related to the possible synergistic effect of aflatoxins in the soil (Sinniah *et al*, 1983).

Schafer and Jacobson (1983) report some toxicological tests on birds using neem extracted or expelled oil with propylene glycol to yield an oral LD_{50} of 1000 mg/kg.

Neem also induces toxic effects in Vector snails (*Melania scabra*) (Muley, 1978), insectivorous fish (*Gambusia* sp.) and tadpoles at 0.4% concentration of extract (Attri and Prasad, 1980).

The most potent compound azadirachtin has been shown to lack mutagenicity against four strains of *Salmonella typhimurium* (Jacobson, 1979). Nimbolide (Uwaifo, 1984) and nimbic acid are also reported to be non-mutagenic. Acute toxicity of the neem extract in mice has been shown to be extremely low (13g/kg) (Okpanyi and Ezenkwu, 1981). Qadri *et al.*, (1984) report subacute dermal toxicity of the neemrich-100 (Tech. - 30% neem oil) in albino rats to show scaling of the epidermis and hyperkeratosis of the stratum corneum in the skin.

In recent years a detailed toxicological investigation of a neem based pesticidal preparation "Margosan-O-concentrate (AI azadirachtin = 3000 ppm) has been done and the product has received a registration by EPA of USA against pests on non-food crops and greenhouse plants. The toxicological data (Larson, 1987) is summarized in Table 1.

Though the above studies point to the neem preparations being much safer, but serious side effects cannot be ignored and should be treated with same caution as medicines or pesticides of synthetic origin. Neem seed oil could be of concern in particular due to its toxicity and hence its use in large amounts may prove hazardous

Table 1: Toxicity studies of Marogosan-O-concentrate.

	Test	Dose
1.	Avain Oral LD_{50}	1-16 ml/kg
2.	Avaian dietary LC_{50} (Bob white quail)	>7000 pp. m
3.	Rainbow trout LC_{50} (96h)	8.8 ml/l
4.	*Daphnia magna* LC_{50} (48 h)	13 mg/l
5.	Acute oral toxicity Rats (14d)	5 ml/kg
6.	Acute dormal toxicity (LC_{50})	> 2 ml/kg
7.	Acute inhalation (albino rats) 4h LC_{50}	> 43 9 ml/l/h
8.	Sensitization(guinea pigs) 6h	non-sensitization
9	Mutagenicity (*Salmonella typhimurium*)	non-mutagenic
10	Honey bees	Benign to honey bees up to 4478 pp. m a.i./ha

(Natvander *et al.*, 1991). Therefore, each preparation needs detailed toxicological evaluation before its commercial exploitation.

Commercialization

It is evident from above discussion that commercial value of neem has been known for decades now and almost every part of the tree is useful in one way or the other. Today neem is being used in a large number of products such as pesticides, toiletry, pharmaceuticals, agronomicals, animal feed, fuel, raw material for industries like paper, plastic etc

Toiletry and Pharmaceutical Products

Neem oil has a substantial use in soap industry. The process for manufacture of soap was patented in India and a hand soap containing neem fatty acids is now manufactured (Godrej, 1975). Margo soap and shampoos made by M/s Calcutta Chemicals, India are being marketed since 1920. A herbal antiseptic liquid soap containing neem extract is manufactured as "Feu Drop" by M/s Nand Kishore Khanna & Sons, Andheri Bombay. Some other neem based soaps available today are "Kutir Neem Soandal" (M/s Thanjavur Sarvodaya Sangh, Tamil Nadu), and

"Parashais Limda Soap" (M/s Parashar Laboratory, Bombay - 77). Margosa neem is a herbal shampoo manufactured by M/s Herbs-n-Leaves, New Soap Products, Hyderabad, A.P. Similarly soaps and shampoos controlling dog ticks and fleas are marketed. Nirmala Neem dog soap is manufactured by M/s Nirmal Enterprise, Bombay - 63.

Pasutone is a cattle feed supplement containing neem leaf powder which kills intestinal worms (Domesto Pvt. Ltd., Vijaywada, A.P., India). Similarly Marguentum forte ointment, a product of Calcutta chemicals is a perfumed neem cream for dermatological use 'Nemlet' is a fly and mosquito repellent and wound dressing that contains neem oil as one of the active ingredients recommended for animal use (Koul, *et al.*, 1990).

Some tablet preparations act as a galactogogue for initiating milk secretion in nursing mothers (Galakol, Charak Pharmaceuticals, Bombay). JK-22 tablets from the same company contain a neem leaf decoction and are recommended for diabetes mellitus of adults, non-ketonic diabetes as well as for cases of insulin fast or insulin sensitivity (Koul *et al.*, 1990). Nimbola by M/s Kee Pharma, New Delhi - 64 is an oral treatment for diabetes control and is stated to have no side effects. However I believe that such preparations should be subjected to extensive toxicological and subsequent clinical trials to adequately demonstrate their efficacy as a commercial product.

Ioquin tablets and injections (J & J Dechance Laboratories Pvt. Ltd.,Hyderabad, India) are being formulated for chronic malaria. A 100 mg tablet contains 18 mg neem extract. Olosyn, a neem leaf preparation is also recommended as a local sedative for external application.

M/s Dabur India Ltd., Ahmedabad, have commercially prepared a pimple cure, "Clean-N-Care" capsules containing neem leaf extract. This discovery is claimed a breakthrough in pimple cure (Parmar and Ketkar, 1993). Curoline is an antiseptic cream manufactured by M/s Chemicure Laboratories Pvt. Ltd., Udaipur - 313001 and marketed by M/s Muller and Phipps (India) Ltd., Bombay-1. This cream contains neem oil and is recommended for sunburns, chapped lips, bruises and cuts, dry skin, diaper rash and after shave "Neemcure" manufactured by M/s Excelsior Enterprises, Kanpur, India is a herbal preparation containing neem oil and neem leaf extract as a part of ingredients and useful as an antiseptic against skin diseases, piles, burns, wounds and injuries.

"Nimodent", a product of Hamdard Co., Karachi Pakistan and neem tooth paste and tooth powder made by Calcutta Chemicals, India are effective dentifrice products (Koul, *et al.*, 1990). Patented extracts of neembark, Silvose-T and Silvose-TRS are used as a toothpaste and a mouth wash respectively. Keimdrat Gmbh, Augsburg, Germany manufactures Dr.Grandel's neem toothpaste containing an extract of neem bark. ORA-neem toothpaste, a product of M/s Nand Kishore

Khanna & Sons, Bombay-34 is an effective toothpaste. Besides leaf extract it contains peppermint and clove oil (Parmar and Ketkar, 1993).

Recently encouraging results concerning the contraceptive effects of neem oil have led to a formula containing the oil that is being manufactured and sold by Excelsier Enterprises, Kanpur, India for intravaginal use (*Neem Newsletter*, 1986, p.37). The commercial preparation "Sensal" contains neem oil (98% w/v), camphor (1.5% w/o), citronella and lemongrass oil (0.5% w/v). Recently a steam volatile, odorous fraction of the oil, coded as Nim-76 is considered responsible for its spermatocidal effect (Riar *et al.*, 1990).

Agrochemical Products

From commercial point of view neem has made its place in pesticide industry in India and abroad. Nearly 30 products are either marketed or are awaiting commercialization in the world. Most of these products are based on either seed extracts or are oil-based. Out of all the products only few have got clearance from various registration committees. Margosan-O-concentrate was the first product from Vikwood Ltd of Wisconsin, USA, which gained Environmental Protection Agency (EPA) registration for the product which contains 3000 ppm of azadirachtin. However, presently W.R.Grace & Co., Washington, DC currently own the rights to this product.

M/s AgriDyne Technologies Inc., USA has developed a neem-based product Azatin-EC for the greenhouse market. This product contains 3% azadirachtin as active ingredient as an emulsified concentrate. Similarly a product in Germany, "Neem Azal", has been developed by Trifolio-M-GmbH and contains 30% azadirachtin - A and some other limonoids too. A formulated product "Neem Azal - F" containing 5% azadirachtin is shown to be ideal pest control formulation in IPM programme.

In India the registered products with Central Insecticide Board (CIB) are "Achook" from M/s Godrej Agrovet Ltd., Bombay - 79 which contains azadirachtin, azadiradione, nimbocinol and epinimbocinol, Margocide - CK and Margocide - OK from M/s Monofix agroproducts, Hubli containing azadirachtin as a major active ingredient; Neemgold from M/s SPIC Ltd., Madras Nimbecidine from M/s T.Stanes & Co. Ltd. Coimbatore and RD-9-Repelin from M/s ITC Ltd., Rajahmundry, A.P. There are other products too (Parmar and Ketkar, 1993) which are still in pipeline to get registration from CIB, India.

Other agro-based neem products are organic-manures. Utilization of neem cake as organic manure began in 1968 (Ketkar, 1976) and yielded positive results. Alcohol extract of the cake when mixed with urea and ammonium sulfate fertilizers, high nitrate levels could be achieved even after 70 days (Sahrawat and Parmar, 1975). A slow rate of nitrification following application of neem cake extracts and neem oil has also been attributed to population reduction of nitrifying bacteria (Koul *et al.*, 1990).

Several field trials with rice have shown that various neem preparations with urea or P_2O_5 + azobactor are very effective in terms of increasing grain yield and protein content of rice (Koul *et al.,* 1990). Field trials of neem cake blended with urea on lowland rice at different locations have been highly successful and are applicable to wheat as well (Sinha *et al.,* 1979). Some recent field experiments with neem have shown its promise in increasing biological nitrogen fixation in wetland paddy fields. By arresting the development of grazing invertebrates as well as nitrifying bacteria, biological fixation could be increased and N-losses reduced simultaneously (Grant *et al.,* 1984).

Neem blended urea has proven useful for sugarcane crop as well (Sharma *et al.,* 1977). From economic point of view 30 kg N + 140 kg neem cake gives high yields with higher net returns (Bawasakar *et al.,* 1980 a,b; Parashar *et al.,* 1980). However, some trials with neem oil have produced better results than with neem cake (IARI, 1983). Similarly in the case of cotton neem cake application (20%) has been beneficial in increasing cotton yield (Kairon and Thorar, 1980).

Major neem-based products for improving nitrogen efficiency are "Neem Manure" from M/s Swastic Chemical Works, Bombay; "Wellgro" from M/s ITC - ILTD DW, Rajamundry, AP; neem extract coated urea and "Nimin" (urea coated with neem triterpenes) from M/s Godrej Soaps Ltd., Bombay - 79; "Jeevan" Soil conditioner from M/s MCDA Agro Pvt. Ltd., Bombay - 1; "Humigold" from M/s Fertiplant Engineering Co., Humigold division, Bombay - 56 and Neem cake mixed NPK from M/s Jaisingpur Mills, Kolhapur, Maharashtra.

Cattle, Sheep and Poultry Products

Neem cake serves as a significant source of cattle, sheep and poultry feed. Extracted seed materials when used as feed mixtures for various laying poultry were comparable to grand nut feed. Similarly neem seed meal rich in protein was palatable to buffalo at a 25% concentration. Neem seed cake feed was eaten at the 10-20% concentration without any effect on milk constituents during lactation (Koul *et al.,* 1990). Gupta and Bhaid (1981) have shown that deoiled neem cake could be incorporated up to 25-50% in a maize diet for sheep without toxic effects. Recently some neem based cattle and animal nutrient constituents have been described in detail (Parmar and Ketkar, 1993).

A formulation developed by Panjarpole Gorakshan Sanstha, Ahmednagar, Maharashtra (ISI, 1977) is stated to reduce the cost of whole feed. "Pasutone" which is toxic to intestinal worms in cattle (M/s Domesto Pvt. Ltd., Vijaywada, AP, India) is also used as a cattle feed supplement containing neem leaf powder.

Fuel

Neem wood has a thermal efficiency comparable to recognized fuel wood is not established but its calorific value is slightly less than that of Enugu coal of Nigeria (Koul *et al.,* 1990) and is reported as 4322.81 Cal/g (Shaheen and Harode, 1987).

Recently Mathur and Das (1985) has compared neem oil with diesel. The blends of the oil with diesel substituting nearly 35% of the latter have been suggested for use without any major engine modification and without any worthwhile drop in engine efficiency.

Neem and Realities

Although the uses of neem are endless nothing is definite in many respects. The greatest impediment to neem's commercial development may be lack of awareness about its promise of becoming a good pesticide or a health care commodity. This has been due to lack of industrial interest. Another difficulty is caused by the fact that the neem trees are scattered around the world and are genetically distinct. This hampers the standardization and, therefore, causes practical problems. To chalk out the regulations no standard of potency has yet been developed, for e.g. the mixture of active ingredients vary with the samples age, the locality, the genetics of the tree it came from and also the handling of the sample. The analytical techniques are tricky, therefore, unreliable.

The conflicting approaches regarding the formulation development vary from crudest to the most sophisticated situation. Of course both approaches are valid but their needs, priorities, costs and objectives are so vastly different that people working at the two extremes may appear to be working at cross-purposes.

There are economic uncertainities such as commercial production of any material derived from the seeds is constrained by nature. There are limitations of seasonal supply and difficult gathering and processing mechanisms. At this point no method for mechanizing the process of collection, storing or handling the seeds is available and nor is it yet known how to carry out these operations. In case of new plantings a delay of several years before the first crop can be collected adds to these uncertainties. Optimization of pesticidal ingredients is still a distant commodity to establish.

Geographical limitations does not allow neem trees to grow just anywhere. They are sensitive to frost and even under dry conditions their growth and yield can be erratic. The short viability of seeds is a problem and within weeks germination rates begin dropping off. It is generally considered that after 2 to 6 months in storage they will no longer germinate. However, some recent observations of seeds that had been stored in France indicated that seeds without endocarp had an acceptable germinative capacity of about 42% after more than five years (NRC, USA, 1992).

Instability is another reality of neem though favourable for quick biodegradation in pesticide use. However, this creates a problem of shelf life of commercial products and use of sun screens becomes a necessity. Neem materials are also sensitive to high temperature and must be stored in shady places.

Although neem has shown every indication of being environmentally safe when used as pesticide; it does not mean that benign and future hazards should be dismissed. A known health hazard may arise as a result of poor handling, such as aflatoxins in seeds due to fungal infections can pass on to usable preparations. In many cases toxicology is still unknown and needs thorough investigations before any commercial preparation is released into the market.

In recent years number of pharmacological effects of neem have been studied but seldom anything has been subjected to rigorous trials with controls. These studies are mostly Indian and other parts of the world look at these claims suspiciously.

Now the question is if neem lives up to its present promise, it will help to control many of the world's pests and diseases and will be useful in agriculture and many day-to-day needs. Accordingly some positive steps which can be taken for promoting the neem's promise could be:-

1. Neem is a good candidate for global tree planting. Whether it will thrive in dense plantation is uncertain but it can grow in certain marginal land.
2. Harvesting neem fruits does not destroy the tree unlike most reforestation species. Thus the use of neem products has the merit of promoting a greening of the earth.
3. In developing neem there is potential for substantial innovation. Assistance is needed to develop high quality ecotypes in terms of azadirachtin content and other desirable traits.
4. Presently the quality of neem extracts varies. The differences are due to variations in seed handling, storage or extraction procedures. Therefore, optimal handling systems need to be developed and standardization up to the international mark is vital.
5. It is well-known now that neem derivatives are promising materials but mode of action in various species is lacking. Such topics deserve greater research attention. Formulation aspects need to be stressed more to keep active ingredients intact.
6. Biotechnology research may also benefit neem by magnifying required traits, examining enzymology and gene expression of active secondary metabolites, gene transfer and genetic mapping of neem's DNA.

Acknowledgement

Author is highly thankful to Mr. C.M.Ketkar, Neem Mission, Pune for interesting information about commercial products. Thanks are also due to Ms. Nishi Mehta for help during the preparation of this article.

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Cultivation and Scope of Improvement in Pyrethrum (*Tanacetum cinerariaefolium* Trev.)

P.N. Pandita

Regional Research Laboratory, Jammu/Srinagar

PYRETHRUM (*Chrysanthemum cinerariaefolium* Vis., Syn., *Pyrethrum cinerariaefolium* Trev; Syn; *Tanacetum cinerariaefolium* Trev.) belongs to family Asteraceae (Compositae), originated in certain warm temperate to sub-tropical regions of the old world: The use of Pyrethrum flowers for insecticidal purpose apparently originated in Iran. The product was known as Persian insect powder and is believed to have been derived from *Chrysanthemum roseum* and *Chrysanthemum carneum* or only of *Chrysanthemum roseum*. These plants were grown in caucasus. In about 1840 a new species *Chrysanthemum cinerariaefolium* was identified in Dalmatia (Part of Yugoslavia) from Italian border in northern Albania and became the major basis of pyrethrum insecticide. The plant was later introduced into European countries. In 1880 it was introduced into Japan and Japan became the principal producer during World War I. This was followed by East Africa during World War II. The plant was introduced into India in the year 1931. According to certain reports pyrethrum has been under cultivation around the world for nearly 160 years (Glynne Jonnes, 1973; McLaughlin, 1973).

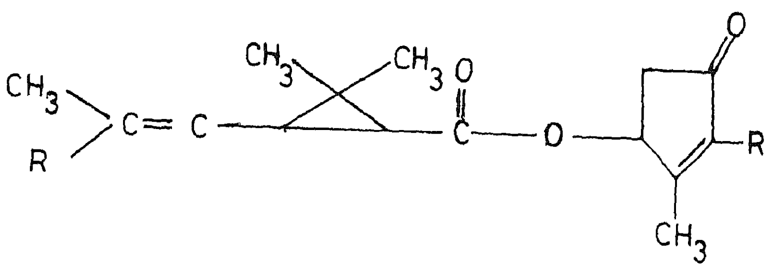
Description of the Plant

Pyrethrum is a perennial temperate plant having daisy like flowers, with lobed leaves. It grows extremely well in high altitude in tropics or low altitude in temperate regions. The plant grows upto 60-70 cm tall with fibrous roots and unbranched stems. The roots penetrate into the soil to a depth of about 2m or more in search of moisture. The flowers are arranged in a close spiral on a convex

receptacle covered by a scale of involucral bracts and are produced on branched leafy stems, rising from a crown of foliage. The outer petals of each flower head are white and surround a central disk of yellow florets. This adds a beautiful sight to the landscape when white flower heads of pyrethrum set in blossom during May-June in Kashmir valley. Because of an effective sporophytic incompatibility system out breeding is the rule (Brewer and Parlevliet, 1969).

Chemical Constituents

The substances in pyrethrum toxic to many insect species are known collectively as pyrethrins. They are mainly concentrated in flower heads and are comprised of six active constituents. Head (1966) has shown them to be



	R1	R	Molecular weight
Pyrethrin I	Me	$CH=CH_2$	328 4
Pyrethrin II	COOMe	$CH=CH_2$	372 4
Cinerin I	Me	CH_3	316.4
Cinerin II	COOMe	CH_3	360.4
Jasmolin I	Me	CH_2,CH_3	330 4
Jasmolin II	COOMe	CH_2,CH_3	374 4

The pyrethrins content of flowers or extractives are usually shown as pyrethrin I and pyrethrin II. The pyrethrin I includes the esters of pyrethrolone, dihydropy-rethrolone and cinerolone with chrysanthemum monocarboxylic acid. The pyre-thrin II includes the esters of pyrethrolone, dihydropyrethrolone and cinerolone with chrysanthemum dicarboxylic acid; flowers also contain pyrethrosin, pyrethrol, lupeol, α- and β-amyrol and taraxasterol; sesquiterp, (+)-sesamin and β-cyclopyr ethrosin; its dihydroderives are chrysanin and chrysanolide.

Uses of Pyrethrum

Pyrethrum is and has been used as an active ingredient of many insecticides and repellents. However, the concentration for using pyrethrum as repellent is 10 times lower than for insecticidal effects. Pyrethrum is mainly used for sanitary purposes in house hold and food shops. In closed rooms it serves against fleas, flies, bed bugs, ants, silverfish and mosquitoes. They are also used to combat ectoparasites of humans and animals, in post-harvest protection of grains, nuts, vegetables, fruits and processed food conservation and in food packing materials together with synergists. The treatment also prevents insect infestation in commercial dry fish. Before the introduction of synthetic insecticides, pyrethrins were also sold for use against cabbage loopers, cabbage moths and some other agricultural pests. But their high cost and low stability in sunlight have restricted their use in agriculture, and are at present commercially used in fumigation of young shoots and late pre-harvest application to combat some soil insects. In short, pyrethrins are highly toxic to a wide variety of insects and cause almost instantaneous paralysis among insects, but are least toxic to human beings. The oral toxicity is reported to vary between 200 to 1400 mg/kg of body weight.

Areas of Production

The major contribution to the world pyrethrum production is from Kenya accounting about 83 per cent (Anonymous, 1983). Tanzania, Ecuador, Rhodesia, New Guinea, Brazil, Taiwan, Japan, U.S.A. and South Africa are the other pyrethrum producing countries of the world. Australia has recently initiated cultivation of pyrethrum on commercial scale. India's contribution to the world market is meagre and cultivation is restricted to Kashmir valley and Nilgiri hills. Even though cultivation trials were reported to be successful from many other places. The Indian requirement is estimated around 300 tonnes per annum which is likely to increase faster. The production of pyrethrum flowers has declined in the country from 100 tonnes (1961-62) to 20 tonnes (1984-85) perhaps due to lack of adequate care in maintenance and improvement.

Pyrethrum Cultivation in India

History of pyrethrum cultivation in India is interesting. In march 1888 a packet of seed of Dalmatian insect powder, received from a correspondent by way of exchange at the Saharanpur garden resulted in some half a dozen plants at Arnigadh. The authorities of the garden without visualising the potential of the plant, simply raised it with other and consigned it to the herbaceous border. The Government of Punjab tried the cultivation at Lyallpur and Murree. Experimental cultivation according to available reports started in Kashmir in 1931, while according to some it was done in 1941 and in Nilgiri hills a few years before the Second World War. Indian Council of Agricultural Research in 1937 considered the production of

pyrethrum with a purpose. The Council recommended that a co-ordinated experiments should be carried out at suitable places to find out if it could be produced economically. Accordingly the Council obtained seed from the Indian Office, London in 1937 and from Dalmatia in 1940 for distribution to different states. Both Dalmatian and Persian plants were introduced, initially. The Persian species was cultivated to a very limited extent in Assam and Murree hills, but, soon it was abandoned as the results of the Dalmatian species appeared more promising. The area under the cultivation of Dalmatian species extended rapidly by 1947-48 in Kashmir, Nilgiris, Assam and several other localities. Attempt to grow pyrethrum in Manali, Research Nursery, Kulu, Palampur, Kumaon, Mysore, Travancore and Kodaikanal gave encouraging results, but, results were unsuccessful from Dehra Dun, Saharanpur, Dharwar, Poona, Ranchi and many other places.

Environmental Requirements

Pyrethrum is a native of Dalmatia, Herzegovina and Montanengra. It is thus a plant of temperate zone, coming from a comparatively dry region which enjoyes warm summers but has marked winter. The crop can be grown in most parts of the temperate regions, where it behaves like other perennials and the flowering is limited to a few weeks in each year. However, when the crop is grown in high altitude tropical zone enjoying Mediterranean climate flowering may be almost continuous throughout the year. This results in much higher yields.

The climatic factors affecting pyrethrum are temperature, rainfall and sun shine hours, and certain inter-actions between these. The plant needs chilling at low temperature to produce good crop of flower buds. The flower initiation is inhibited if mean maximum temperature remains at 24° celsius for a week but temperature below 16° celsius for 10 days, 3 months after the maximum cold, stimulates maximum flowering. Flowering is limited to 3 or 4 weeks in temperate regions, steep slopes of land exposed to high winds are unsuitable for pyrethrum cultivation High rainfall adversely affects growth and yield of pyrethrum, but, thrives best in localities receiving annual rainfall of 80-130 cm per annum.

Soil

After environment soil is the another important factor for pyrethrum cultivation. It thrives, best in well drained loamy soils and can be grown on many types of soils having suitable fertility, high moisture holding capacity. These types of soils are frequently derived from relatively recent volcanic material. Older volcanic or forest soils can also be used for pyrethrum cultivation, provided the soil is sufficiently fertilized and manured especially with phosphate. To realise any increase in flower yield there is need to raise the *p*H of soils to be between 6.5 and 7.5 and to do this the studies call for liming of acidic soils (Sastry *et al.*, 1988). Pyrethrum prefers slightly alkaline soil, but it will still grow well on soils with a *p*H 5.4. Pyrethrum

can be grown successfully on sloping hills, wherever soil conditions are satisfactory. However, these soils require greater fertilization and careful management of irrigation water to get flower yield equal to that received from levelled lands.

Pyrethrum Culture

Pyrethrum continues to be an attractive crop for the small landowner in general, and in the remote areas in particular. Normally, such land owners and farmers often operate in terrain that is nearly impossible to mechanize and their choice of cash crop is very limited. Their agriculture is often no more than a bare subsistence. To such farmers in suitable areas, pyrethrum provides an ideal cash crop and also provides employment for the whole family.

The methods used for pyrethrum cultivation in principal growing countries of the world are discussed hereunder, while basic principles for pyrethrum cultivation have remained unchanged some new techniques have been introduced in recent years in the pyrethrum growing countries of the world.

Selection of Planting Material

Pyrethrum is planted into the field as a seedling or as a split of an old plant, therefore the first step to be taken is to produce planting material. The selection of good planting material is the most effective way of increasing pyrethrum yield. Pyrethrum is a cross pollinated crop, the use of open pollinated seed perpetuates variability in the crop. Hence, detailed attention must be paid to selection and multiplication of best plants, which will help in the improvement and good quality flowers. The planting material can also be raised from hybrid or polycross seed received from a plant breeding station. This will be followed in later years by selection and clonal multiplication. Tuckett (1961) has described an example of selection programme for pyrethrum in detail.

Propagation

Pyrethrum is planted into the field as a seedling or as a split (division) of an old plant. Therefore the first step to be taken is to produce planting material. Direct drilling of seed into the field is impractical, because the seed is very small, having low and uneven germination, further in most cases it takes more than three weeks to germinate and the seedlings grow very slowly.

Propagation by Seedlings

The seedlings are raised in nursery. Since the facilities and services available will vary from place to place, it is not possible therefore, to lay down hard and fast rules on nursery techniques. However, it is raised like usual vegetable seedlings in August-September in Kashmir valley.

After careful working of the ground, the seed is sown in rows or broadcasted on the beds. The seed selected for sowing should be of highest quality, preferably fresh and not in any circumstances more than five years old (Barton,1966; Pandita,1983). Broadcasting produces better results, but it is not recommended because of subsequent weeding problems. Sowing in rows is preferable, with the rows running across the beds to facilitate hand weeding. The seed can be drilled in lines along the rows, where facilities exist and nurseries are mechanized. The seed takes about three weeks to germinate during which time the soil must not be allowed to dry out. Seed germination can be accelerated by soaking the seed in cold water overnight or pre-soaking the seed for 20 minutes in water at 50°C followed by soaking in cold 1.5% solution of $CuSO_4$ for one to three hours. Where soil is moist or irrigation is available the seedlings require three to five or more months in the nursery, before they are ready for transplanting into the field. Seedlings should not be large, that they require sub-dividing or splitting; this practice should be avoided. The evenness of the size of seedlings is obtained by planting seed with an even germination and at controlled rate. Soil fertility should be maintained with the application of a complete fertilizer, farm yard manure prior to seedling. The ultimate aim should be to produce plants in optimum condition for planting out into the field where they will grow at the fastest possible rate.

Vegetative Propagation

Pyrethrum can be propagated vegetatively for field planting and vegetative propagation is to be preferred in absence of any high quality hybrid or polycross seed, available for sowing. The methods employed are either splitting a single plant selection of old material or go in for a composite variety i.e. mix the clonally propagated material of a number of selected genotypes (Pandita and Bhat,1978). Once the splitting material has been identified, certain preparatory measures must be taken and field must be carefully inspected. Plants showing signs of diseases etc. should not be included in the future planting material. Once the planting material has been identified, the next stage is rapid multiplication of the plants by splitting and re-splitting (Brown, 1965) in nurseries for commercial cultivation.

Land Preparation

Soil working depends on the nature of the soil, topography and climatic factors. Majority of the areas selected for raising pyrethrum are either plain or hilly terrains. The soil of entire area is deeply ploughed preferably with a tractor in providing better tilth and soil aeration, which help in the elimination of weed growth. Pyrethrum likes deep well cultivated soil free from hard-pan. The ploughed soil should be levelled pulverised and mixed thoroughly with farm yard manure or compost. The quality of farm yard manure or compost should be added after assessing the original texture of the soil. The planting surface should be made

smooth and besides manure, a fertilizer dose of nitrogen, phosphorus and potash should be applied two or three days earlier to planting.

Local conditions will dictate whether planting is to take place on the ridge or on flat. Normally pyrethrum yields better when it is planted along the top of the ridges. The ridges are prepared as a part of land preparation programme.

Planting and Interculture

The optimum month for planting can be decided according to local conditions. However, the suitable time for transplanting in Kashmir valley is October-November or early spring (Pandita, 1984). Prior to planting out a decision need to be made on the plant population required and hence the spacing between rows and between plants in the row. Different workers from time to time have recommended different row to row and plant to plant spacing, but, experiments carried in our laboratory in Srinagar and in CIMAP at Kodaikanal has shown that a spacing of 60cm × 45 cm produce better results (Pandita, 1984; Sastry *et al.*, 1989).

Pyrethrum plantation should be done thoroughly and frequently. If grass and weeds get a start they are difficult to control. Begin cultivating in the spring as soon as the soil is workable. Cultivate throughout the season as often as necessary to keep weeds down. Discontinue cultivation atleast a month before freezing weather to avoid harming shallow roots of the plants.

Pyrethrum should be cultivated as a pure crop (Pandita unpublished). However, investigations conducted by Ngugi *et al.*,1990 at Kenya to determine the effects of intercropping pyrethrum with maize revealed that flower production increased with decreasing densities of maize in intercropped treatments. Pyrethrins content was not affected by intercropping. However, intercropping treatments gave better returns than pure stands (Ngugi *et al.*, 1990).

Nutrition

Pyrethrum needs an adequate amount of plant nutrients for optimum plant growth. About 100 kg of actual nitrogen is applied during April when crowns begin new growth. Earlier 60 kg phosphorus and 60 kg of potassium is given at the beginning of the season. On South Indian hills the crop responds favourably to higher doses of phosphorus at 120 kg/ha compared with nitrogen or potassium. A great deal of research has been carried out on nutritional requirement of pyrethrum. However, specific rates are determined by soil analysis after assessing the nutrient status of the soil.

Experimental work carried out by Wanjala (1991) showed that triple superphosphate applied at the rate of 200-400 kg/ha gave highest flower yield, but did not seem to affect pyrethrins content. Further, calcium ammonium nitrate at 200 kg/ha and DAP at 100 kg/ha (first year) and at 50 kg/ha (second year) gave

comparatively higher flower yield with higher pyrethrins in Molo, Kenya. Experiments undertaken at Kodaikanal and at several other places revealed that with the increasing rates of phosphorus and potassium, notable significant increase was observed in the flower yield, plant height and diameter of pyrethrum plants. The response of clones to phosphorus and nitrogen was inconsistent and there was no significant difference in dry flower yield between treated and untreated plots. (Ngugi *et al.*,1989; Sastry *et al.*, 1990; Wanjala, 1991 and Salardini *et al.*, 1994). On acidic soils a dose of 45 kg urea, 480 kg rock phosphate, and 30 kg muriate of potash per hectare per annum is ideal for maximum yield. Urea may be applied in two doses, half at the time of planting and rest after three months. Adding lime to the soils also released phosphorus which improves the yield in all, the levels of fertilizers and farm yard manure greatly enhanced the number of tillers and plant height, resulting in higher productivity. There have been some reports of producing depressing effects with the application of nitrogen. Kroll, 1962 has published work on fertilizer experiments from both Kenya and Tanzania. Pinkerton (1970) has described symptoms of all mineral deficiencies. He noted, inter alia, that acute phosphate deficiency caused flower production to cease. Further, it has been reported that plants grown in phosphorus deficient soils respond well to mycorrhizal inoculation. At Kodaikanal inoculation with *Glomus fasciculatum, G. caledonicus* and *Gigaspora marginata* enhanced the plant height, plant weight, number of branches and root length.

Irrigation

The number of irrigations required for crop depends on the time of planting, rainfall distribution during season, type of soil and its ability to store water. In low rain fall areas where the soil is suitable and mean temperatures are adequate, over head irrigation during normal growing period, which in Kashmir, is from April to October can be of great value. Furthermore, irrigation during this period can also have a significant effect on the yield of pyrethrins. In high altitudes where land remains moist for a long time; it could be grown as a rainfed crop. Pyrethrum will not tolerate poor drainage and excess water should be drained off as it adversely affects growth and yield.

Weeds, Pests and Diseases

Weed control is of main importance; because pyrethrum is a slow grower and soil is never completely covered by the crop and inefficient weeding is one of the worst major causes of reduced yields. There is as yet no practical implement able to deal with weeding between the plants, which has to be done by hand constantly and throughout the growing season, particularly during rains. Before the initial planting the land must be thoroughly cleaned of weeds. Grasses which spread by underground stems, are fatal to the plants and have to be removed as soon as they appear.

Weed growth is also stopped by spraying different herbicides. The experiment conducted at Tamil Nadu Agricultural University with "Nitrofen" proved to be superior to all other treatments in reducing the weed population. The efficiency of "Venzar" in the control of weeds was also studied by Ngugi *et al.*, in established pyrethrum fields. The herbicide application at 1.25 kg/ha and 1.50 kg/ha gave satisfactory weed control. However, the application did not have any adverse effect on pyrethrum plants, nor did it effect flower yield and pyrethrins content.

Although this important insecticidal plant can kill a number of insects, diseases and pests have not spared it. The plant succumbs to wilt disease, root rot, crown rot, collar rot, bud disease and nematodes, which not only affect its roots, but, damage its foliage also. Certain bacterial diseases in addition to thrips and red spider mite have also been reported on this crop. However, spraying with dithiocarbamate fungicides and organophosphoric insecticides have given encouraging results in its control. Species of *Alternaria* Nees ex Wallr., *Aspergillus* Mich. ex Fr. and *Penicillium* Lint. ex Fr. were isolated from unsterilized seeds of pyrethrum. Surface sterilization of seed with mercuric chloride (0.1%) reduced the incidence of *Aspergillus* and *Penicillium*. Seed treatment with ceresan (containing 1.5% mercury as acetate) at 1:450 by weight of seeds substantially reduces the incidence of these fungi.

Field Care

Once pyrethrum has been planted, care must be taken to plant seedling or splits deep enough to avoid drying up. This brings us to the other vital point in preflowering maintenance and infilling of young plants; wherever there is mortality. However, the maintenance of plantation has to be continued untill the end of cycle which is usually five to six years from the time of planting. One of the important operation of maintenance is cutting back. This operation is carried out after harvesting the flowers. It is aimed at removing all dead flower stems and unwanted top growth. This prevents physical damage by the old stems to new shoots. It also opens up the top of the plant to allow better and more healthy circulation of air, which in turn helps promote bud formation and helps cut down fungal and bacterial rot. It also helps to prevent the plant from becoming undesirably bushy with lot of woody growth. However,time of cutting back operation is important. The experimental work conducted in Kashmir, Kenya and many other countries of the world has shown that cutting back at the wrong time of the year can adversely affect the total yield, where as doing at the right time will enhance the yield. The actual time chosen varies from country to country and even from area to area. In most of the countries work has indicated that the operation should be performed while production is on the down slope. Before cut, all flowers, including buds should be stripped off; so as to avoid losing them. In Kashmir valley plants flower only once in a year; around last week of May or first week of June. Thereafter, the plant remains in vegetative phase. A number of post-harvest cultural practices are operative here to

induce the plant to flower atleast second time, if not continuously. The experiment to study the effect of cut revealed that the pyrethrum should either be left uncut at the end of flowering or else should be cut upto the level of foliage, which will be about 20 cm above the ground level. Such practice will not disturb the potential of second flowering (Bhat and Pandita, 1974). Cutting back is performed best with a sharp sickle and the object of operation is to remove as much stem and as little leaf as possible while doing this job the plant should not be pulled so as to damage the rooting system.

Hårvesting

Pyrethrum plant acquires a crown diameter of 6 to 12 inches under favourable conditions. A good crop of flowers is produced in the second year. Although some work has been done on mechanization of pyrethrum harvesting, it is probably true that in all the major pyrethrum producing countries of the world, picking is entirely done by hand.

The pyrethrum flowers reach a point where it contains the maximum weight of pyrethrins. If the grower picks before or after this stage, he loses pyrethrins. While in case of small growers it is possible to pick the flowers by involving his family members and go in for selective picking and harvest highest quality flowers, large farm owner has to employ labours for harvesting.

Harvesting by hand is a slow process and poses numerous problems, in particular to the growers in Kashmir valley, because flowers reach and pass the preferred stage of maturity within a very short period. Keeping in view the problems, pyrethrum breeders from time to time have investigated the yield losses in picking of flowers at improper stages of development. Pandita *et al.* (1978) working in Kashmir revealed that it would be preferable to start harvesting earlier; than to delay it and wait for the correct stage of maturity. This way harvesting period will be extended and there would only be a loss of 11.1 to 22.2 per cent pyrethrins, as compared to 23.3 to 50.6 per cent loss in delayed harvesting. Investigations into the yield losses in some pyrethrum clones through picking of flowers at improper stage of maturity were also carried in Kenya by Ikahu and Ngugi (1989). The workers observed that over 64% of the flowers were picked in stage II and III, resulting in great loss of pyrethrins both at farm level and at National level. In another study the workers observed that farmers pick 23% of flowers in stage II, while 28%, 27% and 22% in stage III, IV and V respectively. The results showed that on average there was a loss in pyrethrins of 38.2%, 18.5% and 8.2% when flowers were picked in stage II, III and IV as compared to stage V when the flowers are fully mature.

Selective picking has also been recommended by many workers. This includes picking flower heads at correct stage of maturity. The method entails high harvesting costs and being uneconomical. Further, selection necessitates skilled workers.

An efficient worker can pick 25 to 30 kg of flowers a day; Higher figures have been obtained by exceptional workers. The normal is lower than this. Picking is done, so that the flowers are broken not the stem. The correct way for picking the flowers by hand is that the thumb should be placed on the receptable and the index finger as well as the middle finger on the two sides of the axis below the involucre. The receptable will thus be separated from the axis by a twisting movement causing a clean break at the base of the receptable resulting in the fall of capitulum in the palm of harvester. Picking flowers more rapidly by using hand like a comb always results in bad quality picking with lot of capitula harving lengths of stem attached and many buds included. A picker should usually work with a basket or a container tied or slung on to them in such a way that both hands are left free for picking.

A lot of work has been done on picking machines, but none has perfected a machine that will effectively harvest mature pyrethrum flowers only; leaving the young buds unharmed. The machines developed or used for pyrethrum harvesting are based on basic comb principle, with variable distances between the teeth. Culbertson has made use of some available farm equipment like blue grass stripper for harvesting pyrethrum with a little success. The other implements available on the farm and tried for harvesting pyrethrum, include mower, reaper, grainbinder and cotton stripper. Cotton stripper was found efficient with tall and upright plants but, under adverse conditions the performance was poor. A Danish farmer in Tanzania has patented a machine using a series of travelling pick up bars that rub the flowers off against a freely revolving drum with an adjustable gap between drum and bars. This machine has a little prospects as it breaks the flower heads off in much the same way that they are broken when picked by hand. Yet another machine uses photoelectric cells to differentiate between mature and immature flowers, although it has some possibilities it would probably be too complex to be successfully operated under field conditions. Some stripping devices consisting of a box with row of tenpenny nails with heads removed, set and close together, forming a sort of comb and has been used for harvesting pyrethrum. In another study conducted on harvesting of pyrethrum flowers, stems are cut off with sickles and then drawn through a comb like device either manual or power driven for stripping off the flowers.

Drying

Harvesting should be followed with immediate drying and under no circumstances fresh flowers be left in heaps or in sacks, where they will heat and ferment. They should be spread thinly on the wire trays at density of 3.66 kg/m^2 and dried partly under shade and partly under sun. Shade drying is prefered. Wherever heat drying through driers is available, it could also be employed. The process takes 6-8 hours during which moisture content is reduced to 8-10 per cent, when the flowers are considered worthy for storage.

Pyrethrins become unstable at temperature in excess of 50°C and therefore any drying operation should be aimed at ensuring that the pyrethrins are not subjected to this temperature or over. The effect of temperature on pyrethrins content was studied by many workers on different clones and in all clones it was observed that pyrethrins content decreased rapidly with increasing temperature and reaches lowest at 100°C.

Some times air and sun dried pyrethrum flowers have a higher pyrethrins content, but, experimental work has shown that there is an overall loss of dry matter due to continued metabolism in sun dried flowers and hence loss of pyrethrins.

Packing and Handling

After drying the flowers are packed in gunny bags and forwarded for further processing as soon as possible. Wherever, the facilities are available the flowers are pressed under hydraulic pressure. Pyrethrum flowers, both whole and ground, lose their activity on storage, regardless of the type of containers used for storage. After 20 months storage about 45 per cent of insecticidal activity is lost in dried flowers. Antioxidants apparently retard decomposition to some extent. The flowers, therefore, should be processed without delay.

Yield

The flower yield is variable depending upon the altitude, soil and management. The yield of dry flowers at Srinagar varies from 4 to 5 q/ha and that at Kodaikanal from 2.5 to 3.0 q/ha. In a varietal trial at Kodaikanal certain strains yielded about 6 q/ha of dried flowers. The yield can be improved by the use of good quality seed, cultural practices and cleanliness of cultivation. To be realistic yields should be only quoted as the average annual yield obtained throughout the whole cycle from the moment, when the plant is put into the field, to when it is taken out after five or more years. On this basis, very good plantation that may yield as high as 1000 to 1,500 kg per hectare of dried flowers may average less over the full cycle of plantation.

Utilization

The dried flowers are commonly known as pyrethrum flowers or insect flowers. Powdered pyrethrum has a pleasant and characteristic odour, which is more pronounced in the freshly prepared material. The colour of the ground products depends on its age and the maturity of the flowers. Newly harvested flowers, properly dried and ground, have a bright yellow colour, after storing for some time, or when ground from old or poorly cured flowers the colour is dull and brown. The samples should not contain more than 5 per cent peduncles. About 90 per cent of pyrethrins are contained in the ovary and developing achenes which have many more oil glands, than the rest of the flower, fertilized achenes have more than the unfertilized ones.

Pyrethrum is used as an insecticide in number of ways, in the form of powder, sprays, aerosol coils, creams and ointments. The pyrethrum marc, i.e. the grist from flowers, left after extraction of pyrethrins is used in making mosquito coils. It is also an excellent filler for insecticidal dust against pests, especially the beetles. The marc is also employed as a feed for livestock which find it palatable; often molasses and sometimes fenugreek is added for improving palatability. It contains protein, 13.05; carbohydrates 55.7; fibre, 23.0; oil,0-5; ash,7.1% and pyrethrins in traces.

Breeding and Improvement

Pyrethrum has been under cultivation around the world for nearly 160 years (Glynne Jones, 1973; McLaughlin, 1973). The plant is still in its early stages of genetic improvement. For pyrethrum to continue as a crop that can produce increasing returns to its growers, ways must be found to increase the yield per unit area of land per annum. The most obvious way of achieving this is by the production of plants with high yielding characteristics and flowers with high pyrethrins content.

Pyrethrum being a minor crop has not received much attention in the past and it is only during the past decade some serious work has been done on pyrethrum breeding and improvement. The initial attempt was on the development of hybrids (Kroll, 1958) which was followed by release of superior clones (Contant, 1963). Parlevliet (1974a, 1974b) has indicated its potential.

A population improvement programme was initiated in Kashmir, in the early 1970 (Bhat and Pandita, 1977). The programme was started on the population already under cultivation in drug farms of the forest department. The back ground of this material is briefly described here. Pyrethrum was introduced into Kashmir valley nearly 60 years, ago, when it was grown experimentally at the drug farms. Since then this population along with other introductions has been growing together at the drug farms without any conscious selection. Any new plantings or replacement of the old ones were made with the seed harvested from the population maintained under random mating. Substantial magnitude of variability was encountered in almost all the characters including pyrethrins content.

Pandita and Bhat (1984); Singh *et al.* (1987a, 1987b); Pandita (1988 unpublished) studied the genetic variation of several economically important yield components of number of selected clones. The range of variation observed was very high for plant height; bush diameter; number of flowers per plant flower diameter; 100 flowers weight, flower yield and pyrethrins accumulation.

Parlevliet (1974a) in Kenya also studied the genetic variation of several commercially important characteristics. The range of observed variation was very wide for fresh flower yield, flower size, number of flowers per stem, pyrethrins content, lodging resistance and the pyrethrins I/pyrethrins II ratio and quite narrow for the dry matter content of flowers. Populations to select from were obtained by

crossing two single and one polycross. The single crosses showed, for the three characteristics measured, pyrethrins content, flower size and Py.I/Py.II ratio, a considerable transgression. The progeny means were very similar to mid parent values, which indicate a polygenic inheritance for these traits. The variation of the polycross for these traits was not greater than those of the single crosses suggesting a very high level of heterozygosity in the parental clones, it is in fact possible to reconstitute a large part of genotypic variation from only a very few single crosses.

The major pyrethrum producing countries of the world have profitably exploited the variation in the improvement of the crop and developed several improved strains for commercial cultivation (Kroll, 1957; Parlevliet,1969; Parlevliet & Contant, 1970; Parlevliet, 1974a). The first step in the pyrethrum breeding work is the selection of individual plant which show desirable features. Such plants are divided into splits and planted in clonal rows for observations. Most of these single plant selections are made within collections of hybrids. As pyrethrum is a perennial crop, it is essential to test the performance of all materials over a period of three years and only those plants are retained which give satisfactory yields. These populations must be maintained, and simultaneously improved by using the selected individuals as parents for the next generation. The most suitable mating systems for this purpose are random mating and phenotypic dissortative mating i.e. the mating of plants which are dissimilar for the selection character(s) in question. By means of both systems, but particularly by dissortative mating, the occurrence of extreme phenotypes is limited and this tends to prevent valuable blocks of linked genes from being lost from the population. This is of great importance in pyrethrum. Further, it is very important that sufficient plants should be retained in each generation and that all these plants should be used as parents for the next generation. However, where the most rapid results are required in the field on a comparative large scale, it will be necessary to use most of the selected material initially for the production of polycross seed. As many good clones (not less than five) are put into seed production, which is likely to be the most effective for the purpose of improving pyrethrins content rather than yields from commercial plantations. All this work can be done comparatively easily on most commercial plantations where conditions are reasonably controlled.

Pyrethrum is essentially a cross pollinated crop and seedling populations exhibit a considerable genetic and phenotypic variability for many characters. The performance of the best individuals in such a population far exceeds the average performance of population as whole. As pyrethrins content and flower yields are inversely related, individuals with a high expression of both characters are of a very rare occurrence. The genetic fixation of such desirable type is one of the greatest problems in the breeding of cross pollinated crops. It is, therefore, clearly desirable to effect this fixation by vegetative propagation as recommended from time to time by different workers (Brown, 1965; Grewal *et al.*, 1978; Pal *et al.*, 1985) of outstanding plants.

Crosses of different types may also be made to exploit the possibilities of hybrid vigour and specific combining ability. Since the commercial use of varieties grown from seed is of limited justification, in view of the fact that pyrethrum plant can be easily propagated from splits. The importance of this asset has been realised by several workers (Drain, 1934; Chamberlin, 1947; Osbourn, 1961; Cormack, 1935; Pandita *et al.*, 1978).

The possibility of inbreeding is being also investigated but the use of this method depends largely on the discovery of adequate means of over coming incompatibility.

Investigations have revealed that pyrethrins percent was not significantly correlated with any of the morphological trait. However, plant height showed highly significant correlation with tillers per plant, flowers/plant, fresh flower weight, dryflower weight and diameter of the flower. It was also observed that more tillers/plant had a tendency to reduce the flower diameter. The other correlations of interest were that, heavier fresh flower produced flowers with wider diameter and higher moisture percent.

Many workers engaged in breeding for qualitative characters have been striving hard to get hold of such a correlation which would alleviate the burden of lengthy chemical analysis. In pyrethrum, such an attempt has also been made by different workers from time to time, but none of these studies proved of benefit in selecting strains for high pyrethrins content, without going in for chemical analysis. Notcutt, *et al.* (1955) observed a significant positive correlation between number of oil glands and pyrethrins content. Muturi (1967) and Mwakha (1970) showed a negative correlation between pyrethrins and flower yield which could not be established by Parlevliet (1974a). However Parlevliet (1974a) observed negative relations between flower size and dry matter content of the flowers and flower yield. He could not get any confirmation on the suspected negative relations between flower yield and pyrethrins content. Pandita and Bhat (1986) after refinement in the measurement, found pyrethrins content significantly correlated (p. 0.05) with the width of disc florets. Further, it did not reveal any statistically significant association with any other character. As regards the correlation of other traits, more side floret length indicated more central floret length and width of side and central florets.

Singh *et al.* (1987) observed high correlation in flower diameter and pyrethrins content on one hand and flower number and flower yield on the other, there was no relationship between flower yield and pyrethrins content. The direct influence of bush diameter, number of flower per plant, flower diameter and 100 flowers weight on pyrethrins content, is positive, while that of plant height and flower yield was negative. Further, Singh *et al.* (1988) estimated parent offspring correlations and found them highly significant for number of flowers per plant, flower weight and pyrethrins content as well as yield.

In a study in Australia on genotypic and phenotypic correlations in 15 clones and 2 seed populations, it was observed that flower yield was more dependent on the number of flowers per plant than on 100 flowers dry weight. There was a negative correlation between the number of flowers per plant and 100 flower dry weight. These results indicate that large genetic improvements are possible in fairly short time.

The current pyrethrum breeding programmes are directed towards exploitation of exotic and local germplasm in improving and stabilising the yield potential. Hence proper choice of parents which can nick well to produce superior off-springs, is essential for rapid success in any conventional hybridisation programme. Diallel technique of analysis provides genetic information on the inheritance and behaviour of quantitative characters associated with yield and yield components. Further, evaluation of combining ability of genotypes helps in identification of suitable parents for further exploitation. The general combining ability is based on additive gene action, whereas specific combining ability is dependent on non-additive type of gene action. Studies have indicated that varieties good in per se performance, may not necessarily produce desirable progenies when used in hybridization.Hence knowledge of combining ability of parents can be of immense value in predicting the performance of their crosses.

In combining ability studies by Pandita *et al.* (1986) for characters like pyrethrins content, dry flower yield, flowers/ plot, moisture content, buds/plot, flower weight and pyrethrins yield high ratio of specific combining ability to general combining ability was observed which indicated predominance of dominance gene action as compared to additive gene action. Contrary to the above results Parlevliet and Contant (1970) while studying general combining ability effects for pyrethrins content and flower yield in 22 clones determined by means of Polycross showed that the performance of single crosses is caused predominantly by general combining ability of the parent clones. Therefore, single crosses offer no fundamental advantage over synthetic varieties. So that for practical reason, the latter are preferred. At the same time it is recommended to practise reciprocal recurrent selection as this will exploit, the general combining ability in the initial stages of selection and the specific component increasingly in latter selection. These studies have given useful informations about the choice of parents for further breeding, whether it is the general combining ability as revealed by Parlevliet and Contant (1970) or the specific combining ability as reported by Pandita and Bhat (1986) can be resolved by detailed investigations on the genetic architect operative in the inheritance of different economic traits of pyrethrum.

It was found that environment exerted a significant influence in the expression of weight, number of dry flowers/plot and moisture content. Pyrethrins content was observed to be a stable character and the cultivar with high content of pyrethrins yield consistently and uniformly over a wide range of climate. Ikahu and Ngugi (1988) working on the yield assessment of newly developed pyrethrum varieties in

different ecological zones of Kenya found environmental factors affecting flower yield and pyrethrins content. An interesting feature of environment was observed in pyrethrum grown at very high altitudes, where temperature drop regularly near freezing, is that plants tend to become vegetative in habit and turn partially blind.

The knowledge of the heritability of the trait is important for the breeder, because it gives him an idea of the extent of genetic control for the expression of a trait and is defined as the ratio of genetic variance to phenotypic variance, Allard (1960). The estimates apply only to a particular population growing in a particular environment and may also change with age. The heritability estimates of pyrethrum were estimated by Parlevliet and Contant (1970); Pandita (1979); Singh *et al.* (1988) from parent-offspring regression, variance components derived from the analysis of variance; polycross progeny data and single cross data. All the results signified high heritable nature of number of flowers, flower weight; pyrethrins content; flower yield and lodging resistance, which agree with the observed facts that phenotypic selection for these traits is very effective.

For many years work in Kashmir, Kodaikanal, Kenya and several other places was based on the production of new varieties which were either first generation (F_1) seed harvested from selected clones or clonal planting material of a single clone. However Pandita *et al.* (1978) recommended the release of composite varieties of a number of selected genotypes for commercial cultivation. Pyrethrum breeders from time to time in several countries, such as Kenya, Japan, Australia and India have developed vegetatively propagated clones and seed strains by selection and hybridization like "Conagen"; Shirayuki, HYPY, Hansa, SL-781, SL-71564, SL-882 and several other strains which produce numerous flowers with large centers erect in growth habit, synchronous, with varying maturity periods and high pyrethrins content (more than 2.0 per cent).

Tissue Culture

Considering the long time required for breeding new pyrethrum cultivars/strains using conventional methods, a rapid multiplication of tested genitors for combining ability is of high interest. Since pyrethrum plants can be propagated easily by splits; selection and multiplication of superior clones have been indicated to be better method of improvement than production of hybrid. The increased interest in the cultivation of this plant demands that a large number of plants be made available for commercial cultivation. Only the '*in vitro*' technique has been proved to be a suitable method for cloning of special selections of parent plants or cultivars in large quantities when compared to vegetative propagation of the selected plants by splits and multiplication from shoot cuttings. Complete plantlets have been developed from explants of the hypocotyl, leaf blade, the peduncle and sections of capitulum and petiole (Roest and Bokelmann, 1973; Grewal *et al.*, 1978; Pal *et al.*, 1985; Sarker *et al.*, 1991). These processes are controlled by various factors. These

factors are related to properties of the explant, the nutritional/ hormonal composition of the medium or substrate and the climatic conditions to which the explant is exposed. Anatomical observations have also revealed that in these processes successive stages can be distinguished, such as intiation and development, before flower heads, roots or shoots are produced. Some factors had an effect during initiation, which differed from that during the stage of development. Hence an optimum course of these processes can only occur when specific conditions are provided in every stage.

Pal *et al.* (1985) initiated calli development from leaf and petiole explants of chromosomally homogeneous population of *Chrysanthemum cinerariaefolium*. Direct shoot regeneration was obtained from the petiole and leaf explants. Sporadic shoot differentiation was also observed from the leaf grown calli and roots readily developed when transferred to rooting medium. Cyto- differentiation at different passages and also in two different media from short term cultures i.e. upto 5th passage showed consistent diploid (2n = 18) chromosome number. In contrast, polyploid cells appeared in long term cultures. Profuse mitotic abnormalities were observed when calli were maintained for longer period. Leaf regenerated plants were homogeneous and identical in terms of ploidy and chromosome morphology to that of the donor plant. However, plants with a range of chromosomal populations were raised from the petiole explants. This investigations has shown that petiole explants could be exploited to raise new cytotypes of pyrethrum plant.

Further Paul *et al.* (1986) developed a procedure for regeneration of pyrethrum plant (*Chrysanthemum cinerariaefolium* vis) from the callus cultures. Shoot morphogenetic capacity of two culture lines C_5 and C_9 of *Chrysanthemum cinerariaefolium* viz. with higher pyrethrins content exceeds that of culture line C_{10} which was equal in amount of pyrethrins to that of leaf explants. Culture line C_5 with comparatively high pyrethrins content excelled over the other two culture lines in its regeneration ability. Loss of totipotency in the respective long term cultures is accompanied by concomitant decrease in pyrethrins synthesis. However with petiole derived cultures pyrethrin yield was higher in leaf derived cultures of *Chrysanthemum cinerariaefolium*. Highest pyrethrin accumulation was recorded at the second passage of callus growth and the content declined over time under uniform cultural conditions irrespective of the explants and genotypes used. Pyrethrin production of four culture lines established from SL-821 remained quite stable with regard to productivity. All the six ester components of pyrethrins were present even at the twelfth passage of both leaf and petiole derived cultures of the two kind of source plants (Sarker *et al.*, 1991). Grewal *et al.* (1978) studied that the plant part used to imitate the culture appears to have little infleuence on pyrethrum production capacity.

The influence of chemical, physical and biological factors on the production of secondary metabolites has been reported in pyrethrum from unorganized cultures earlier (Zeig *et al.*, 1983; Pal and Dhar 1985, 1986). Zeig *et al.*, 1983 in an extensive

study have shown that high yielding genotypes of *Chrysanthemum cinerariaefolium* produced higher yielding callus strains and the various explants have little or no difference on amount of pyrethrins synthesis. The investigation conducted on explants of leaf and petiole of two selected clones of *Chrysanthemum cinerariaefolium* vis HSL - 801 and SL 821 which yield 1.5 and 1.0 per cent pyrethrins in their flower heads. The pyrethrins content of leaf derived calli of both the clones mostly remained higher than that of comparable petiole derived calli. The yields of the investigated cultures were maximum during the second passage except under certain treatments. Highest pyrethrins content was recorded in leaf cultures of clone HSL - 801 under treatment with 2,4-D and IBA, both at 0.5 mg/l concentration. Even though the pyrethrin content of culture declined in subsequent passages it remained as high as 370 mg per cent till 10th passage. The comparable culture raised from the low yielding clone viz. SL-821 also yielded significantly higher amount of pyrethrins i.e 330 mg per cent in both the clones: No positive correlation could be established between the pyrethrin yield and the growth rate of calli derived from leaf or from petiole explants. However, it may be stated that leaf of *Chrysanthemum cinerariaefolium* is a better vegetative explant than petiole in relation to pyrethrin yield.

Economics of Cultivation

The current market rate is about Rs.5500/q of dry flowers. A reasonably good crop grown under given agrotechnology is expected to produce about 400kg/ha of dry flowers valued at Rs.22,000. It requires an average expenditure of about Rs. 7,000/ha in the first year and Rs.9,000/ha in the subsequent years. Thus it has a possibility of becoming a cash crop in the hilly areas where soil and climatic conditions are suitable. The profits will increase with the use of new strains.

Trade

In the absence of sufficient statistical information, an assessment of the world market for pyrethrum is not possible. However, the developed countries have been the major importers and users. The Indian requirement is estimated at 300 tonnes per annum. The production in Kashmir and other parts of the country is around 20 tonnes of dry flowers. India imports large quantities of pyrethrum from Kenya. The import of pyrethrum in our country in different years is given in Table 1.

However, during 1983-84, India re-exported pyrethrum to the tune of 19,000 kg valued of Rs. 1,226,455 to USA.

Prospects

The production of pyrethrum is much less than the potential world demand. The reasons of this are ecological and economic conditions in the producing countries. The limited availability of pyrethrum excludes it from application at large scales.

Table 1– Import figures of Pyrethrum

Year	Quantity (kg)	Value (Rs)
1982-83	80,800	2,348,626
1983-84	108,900	2,616,909
1984-85	180,000	4,424,881
1985-86	62.960	1,558,101
1986-87	186,000	4,284,375
1987-88	-	-
1988-89	-	-
1989-90	-	-
1990-91	-	-
1991-92	12,60,000	5,995,211

Further, during the last few years the demand for pyrethrum has increased strongly. due to growing public alarm over dangerous side effects and the serious concern of natural imbalance resulted from large scale use of synthetic insecticides and other chemicals. This dilemma has made it essential that such methods of controlling insects be found out which do not create complications and are acceptable to both public and health authorities. Hence plant products/components with insecticidal properties are becoming main agenda of the scientific concern because they are comparatively safer for the environment. The pyrethrum is one of such plants. To conclude pyrethrum insecticides, due to their selective mode of action could be safely used in integrated pest management, their relatively low mammalian toxicity is also beneficial. People are unaware about the usefulness of these products and thus concerted efforts are required to popularise pyrethrin and Pyrethrum products.

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*Potentialities of Pyrethrum Cultivation in South India

N.Kumar & M.Kannan

Horticultural Research Station
Tamil Nadu Agricultural University
Kodaikanal - 624 103, Tamil Nadu

Introduction

PYRETHRUM, *Chyrsanthemum cinerariaefolium*, viz., is an important medicinal plant yielding commercial plant based insecticide and is occupying unique place on account of its cheapness and wide applicability. The crop is reputed for its insecticidal activity by virtue of an active principle 'Pyrethrin' lying in the flowers. Pyrethrum is much preferred to synthetic insecticide as it is one of the safest insecticides known. It has a very low mammalian toxicity and it rapidly gets metabolized if swallowed accidentally. Being a safest insecticide, it can be used toward off insects damaging food grains and against household insects. The other important species yielding pyrethrins are *C. coccineum and C. marschalki.*

Pyrethrum was originated in Dalmitian region but Japan captured the world market during World War I. Kenya introduced the crop in 1928 and its cultivation received an impetus during World War II. The plant is now cultivated in many parts of the world. Kenya is the chief producer currently.

In India, it was introduced in 1931 in Kashmir and in 1942 it was tried in several parts of India. The commercial cultivation was taken up in Kashmir alone and the awareness for raising this crop now spreads to South India hills also. Nilgiris and

* Revised and updated

Kodaikanal hills of South India, enjoying an akin climatic conditions as that of Kenya, being situated near equator, provides ideal conditions for its production. Many experiments were conducted at Kodaikanal under the aegis of All India Coordinated Research Project for Medicinal and Aromatic Plants of the Indian Council of Agricultural Research to evolve practices to increase the production and quality of pyrethrum.

Botany

The plant grows to a height of about 80 cm and is bushy and rather woody at the base. The lower leaves are alternate, petiolate and divided into lobed and dentate segments on the flowering stems. The leaves are smaller and have only 2 or 3 segments. The solitary flower head carried on a striated stalk has 2 or 3 rows of lanceolated hairy bracts forming the involucre, a single row of cream or straw coloured ligulate florets and a disc of numerous yellow tubular florets.

Pyrethrin Content

About 95% of insecticide activity lies in the flowers and comes from mixture of six chemical constituents. Pyrethrin I, Pyrethrin II, Cinerin I, Cinerin II, Jasmoline I and Jasmoline II are collectively called 'pyrethrins'. Total pyrethrin content in south India Hills varies from 0.042 to 1.9% but average being 1.19%. In Kenya average pyrethrin content is 1.4 and the highest content of 2.1% has also been recorded. The pyrethrin is distributed in different parts of the flowers but is more in achenes (93%), while the disc florets and ray florets have only traces.

Parts of the flower	Composition of flower by weight (per cent)	Percentage of total pyrethrin
Achenes	34.2	92.4
Receptacles	11.3	3.5
Involucre scales	11.5	2.0
Disc florets	25.8	Trace
Ray florets	17.2	Trace

Floral Biology

Vadivel and Sampath (1981) studied the floral biology in pyrethrum and found that an average of 54.1 days are required for a well developed enclosed bud to reach the disc florets fallen stage which is the ideal condition for collection of seed. It is also evident that maximum percentage of the buds opened between 6.00 am to 9.00 am

and another emergence between 9.00 am to 11.00 am. The stigma in pyrethrum was receptive from a day prior to opening of the flower to two days after opening of the flower with most receptive condition on the day of opening of the flower (72.00%).

Vadivel and Sampath (1981) further reported that pyrethrum, which is a predominantly cross pollinated crop, recorded a reduction in seedset, weight of the seeds and pyrethrin content when the flowers were selfed. There is a slight increase in number of seeds and weight of the seeds per flower in artificial pollination over open pollination. However, variation in pyrethrin content between open pollination and artificial pollination is meagre.

S.No.	Mode of pollination	Pyrethrin content (%)	Number of seeds set	Weight of the seeds per flower per flower (mg)
1.	Self pollination	0.895	179.0	147.6
2.	Open pollination	1.107	237.6	205.6
3.	Hand pollination (Artificial)	1.109	266.2	254.2

Pollen viability ranged from 76.14% to 94.12%. The pollen germination percentage varied from 44.51 to 61.40 per cent in different concentration of sucrose solution, i.e. 10 to 25 per cent.

The flower head, being the economic trait, the association studies conducted by Vadivel *et al.* (1981) recorded that the traits like flower depth, flower diameter, individual flower weight and number of flowers per plant are the important characters which should be given due weightage while exercising selection.

Climate

Pyrethrum thrives in cool, dry climate of south Indian hills (above 1800m M.S.L.) and Kashmir valley. Low night temperature favours the flower production. It is sensitive to frost in its early stages. An annual rainfall of 100-150 cm, distributed over a period of 8 to 10 months is desirable. These conditions are prevailing in the hills of Kodaikanal and Nilgiris. Kodaikanal is situated at an elevation of 2280 m above M.S.L. with 10.2°N latitude and 77.5°E longitude. The annual average rainfall is 1650mm in 180-200 days. The maximum temperature ranges from 15.9°C (November-December) to 26.7°C (May) and minimum temperature from 1.2°C (December-January) to 13.5°C (May-June). Ground frost occurs during December-January in places at higher altitudes.

Soil

Pyrethrum can be grown in a wide variety of soils but good drainage is essential. In South India, it is grown in Black loam where the proportion of clay is slightly higher and red sandy loam which is lateritic. The *p*H of the soil ranged from 5.5 to 5.7. The N content was low (94 kg/ha) and potassium 14 to 22.5 kg/ha. On the Nilgiris, the crop was grown in poor extremely acid soils of *p*H 3.0 to 5.1 (Nair & Raghava, 1955). Liming the soil at 50% of the soil requirement is an ideal practice to alleviate the problem of locking up of phosphate in acid soils either as iron or aluminium phosphate and thus increasing the yield. The establishment and growth are normally better in sandy loam with soil thrown to ridges than on black loam planting on flat ground.

Nursery

Standard nursery beds of 6.0 m length, 1.2 m width and 10 to 15 cm height are formed and mixed thoroughly with well decomposed farm yard manure, sand and shola soil. Four to five such beds may be required to produce sufficient seedlings for planting one hectare. 50g of seeds are sown per standard bed. Pretreatment of seeds with cold water for a period of 6 to 12 hours results in good germination. Floating seeds may be removed as they usually result in very poor to nil germination. Mohandass and Sampath (1983) observed that pyrethrum seeds soaked in Gibberelic acid at 50 ppm improved the germination percentage from 58.0 (in control) to 76.00 per cent. Besides, the treated seeds germinated earlier than the untreated ones. After sowing, the seeds are covered with a thin layer of earth and pressed gently and a layer of grass (5 to 8cm thick) is laid directly on the soil. The beds should be watered daily from a very fine rose can. Germination starts in about 8 days and is complete in about 15 to 21 days. As soon as germination is more or less complete, the grass cover should be removed. The young seedlings are highly susceptible to damping off and if any sign of this is observed before the germination is complete, the grass cover should be removed and sun light allowed to fall directly on the bed. The seedlings will be ready for transplanting within 60-75 days.

Pyrethrum can also be propagated from 'splits' obtained from older plants. The advantages in planting splits over seedlings is that they establish themselves much quicker, especially if the planting is followed by a spell of wet weather and they come to production earlier than seedlings besides producing higher yields during the first year. Seedlings, however, survive much better than splits in dry weather.

The efficiency of vegetative propagation through splits and crown cuttings as compared to seed propagation when assessed (Arumugam & Dakshinamoorthy, 1979), the results showed that the seedlings and splits established well after planting compared to the crown cuttings. There was no perceptible difference in the flower yield between the seedlings and splits though the splits were earlier in flowering by 15-30 days.

Preparation of Land

The land is ploughed or dug thoroughly twice or thrice to a fine tilth. Slopping ground is well terraced or graded contour trenches are formed at suitable intervals. In the Nilgiris, in addition to graded contour trenches, uncultivated grass belts of 1.5 m to 2.0 m width are left alternating with cultivated strips of 3.2m to 9.0m width to prevent any soil wash.

Transplanting

April-May planting immediately after the premonsoon showers is the best time for transplanting in South Indian hills. Seedlings with atleast 3 pairs of leaves are carefully lifted from the nursery beds and taken to the planting area in baskets. The planting holes must be dug deep enough to receive the whole root without the roots being twisted upwards. The spacing normally adopted is 60cm between rows and 30 to 45cm within the row. After the roots have been inserted as straight as possible, the soil must be pressed firmly around. It is advantageous to cut back newly planted pyrethrum when about three stalks have developed, because this encourages the seedlings to shoot forth with great vigour. The seedlings should establish themselves under normal climatic conditions in about a month's time.

Varieties

There is no named variety available, however, RRL, Jammu has released one composite variety viz. C. 793 which is high yielding and having high pyrethrin content. Clonal selection programme undertaken at Horticultural Research Station, Kodaikanal led to the identification of a high yielding type viz. EC 113408-37/4, (Kumar & Sampath, 1981).

Manuring

Pyrethrum responds to phosphatic fertilizer applications. In South India, a fertilizer dose of 20 kg N. 120 kg P_2O_5 and 20 kg K_{20} is normally recommended for a hectare plantation (Kumar *et al.*, 1981). They found at Kodaikanal that there was a good response to higher doses of P_2O_5 at 120 kg/ha compared to 40 or 80 kg. Flower yield by number and weight were higher (by 9 per cent and 11.1 per cent respectively) in plants receiving 120 kg dose than 80 and 40 kg.

In Kashmir, application of 60 kg N and 40 kg P_2O_5 is recommended per hectare. In Kenya, small quantities of phosphates are applied in and around the planting hole to increase flower production. Elaborate experiments are also reported in Kenya from 1945 to 1960 on the use of different forms of phosphate as super, rock phosphate and kenaf and different doses of phosphorous and on

placement of fertilizers. It was found that especially on the more leached and exhausted soils of the high altitudes, Phosphorous was almost a necessity. Soils which had given ready response to P_2O_5 were usually low in base saturation and *p*H value (Kroll, 1964). Studies on the inoculation of seedlings with VA mycorrhiza revealed that flower production can be significantly increased (41.43% over control) (Anon, 1986).

Trials conducted at Nilgiris have indicated no response to manuring in poor, extremely acid soils. In Dalmatia, cultivators do not apply fertilizers in calcareous alkaline soils.

Weeding

The flower production in pyrethrum is greatly influenced by weed competition. Manual weeding is best done by handpulling at regular intervals and before the plants flower. Normally two weedings are done during the year of planting; the first weeding early in July and the second weeding after the South-West monsoon is over. i.e. by September-October. From the second year onwards, it is necessary to do three weedings a year, the first weeding in July, the second in September-October and the third in June.

Chemical weed control studies revealed that the application of TOK.E. 25 (Nitrogen) at 1.0 litre/ha as pre-emergence spray plus hand weeding 45 days after chemical spray was found to be effective in controlling the weeds to an extent of 79 per cent over unweeded plots. The chemical weeded plots resulted in a higher yield (687.06 kg of dry flower/ha) as against in control plants which recorded only 262.48 kg/ha dried flowers (Srinivasan *et al.*, 1982).

Picking

The keeping quality of pyrethrum flowers and the pyrethrin content depend entirely on stage at which the flowers are picked. Immature flowers and over mature and full blown flowers contain less pyrethrin and it is therefore absolutely necessary to pick the flowers at the correct stage; i.e. the flowers have atleast 3 to 4 rows of disc florets open. Harvesting interval varies from 7 to 18 days in a locality. In Kenya, the flowers are picked at intervals of 10 to 14 days, in Dalmatia once a week and in Japan at intervals of 15 to 18 days. In South India, it is picked at 14 days interval.

The flower-heads are picked from the stalks using the thumb and index finger by a gentle jerk without causing any injury to the plants. The flowers are usually carried in baskets so as to allow aeration. If the flowers are gathered in a closed vessel and compacted, the pyrethrin may get decomposed due to the heat produced.

The flowers may be dried in the sun or in specially constructed kiln driers. Sun drying will usually take about 4 days and is possible only in dry weather and if the acreage is small. During wet weather, and if the quantity of flowers picked is large, it is necessary to have kilns for drying.

Yield

Pyrethrum plant starts flowering in about 6 to 9 months from planting in South Indian hills and also flowers throught out the year (Table 1).

Table 1 – Effect of seasonal variation on flower production and pyrethrin content in pyrethrum

Month	No. of flowers per plant	Dry weight per flower (g)	Total pyrethrins content (%)
January	23.23	0.292	0.950
February	17.61	0.267	0.850
March	41.22	0.244	0.994
April	25.77	0.188	0.862
May	22.58	0.306	0.830
June	24.26	0.293	0.872
July	28.54	0.441	1.120
August	22.48	0.316	0.930
September	17.68	0.171	0.800
October	15.66	0.205	0.960
November	21.90	0.245	1.050
December	18.18	0.321	1.000

But it can be seen that flower production is not uniform throughout the year and is generally more when clean weather prevails. Lesser flower production is often met during rainy months (Mohandass *et al.*, 1981).

It starts flowering only during second year of planting in Kashmir, that too, only for 3-4 months during summer. The average yield in Kashmir is about 250 kg/ha while in South India, it varies from 180 to 400 kg/ha. Normally, pyrethrum plantation can be retained for 5 to 6 years and under South Indian hills, a period of 3-4 years is found to be economical.

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*Agar Industry — Past, Present and Future

K.Rama Rao

CSMCRI-Marine Algal Research Station
Mandapam Camp-623 519, Tamil Nadu

THE term agar is abbreviated from agar-agar of Malayan origin which means a jelly obtained from certain red seaweeds. A jelly is produced from the seaweeds when they are boiled and the resulting liquid is cooled. The discovery of agar dates back to 300 years with a traditional story in Japan. One day the Emperor of Japan was marooned in a snow storm and took refuge in an inn. The inn keeper Tarazeoman Minoya served some seaweed jelly to the Emperor and threw the remainder outside into the snow. The next morning he discovered quite a new frozen substance from what he threw out, which was good. The frozen jelly turned into a dry papery translucent subtance, which the inn keeper discovered could be reconverted into jelly. And it was first produced commercially in Japan in 1670. And thus it became the first seaweed product to become an important item of commerce and Japanese since then lead in its production. However, due to its acute shortage during the Second World War, agar has been produced in substantial amounts by other countries such as Britain, Denmark, Australia, New Zealand, Chile, Spain, Italy, Canada, South Africa, United States, USSR, China including India also.

Marine algal species of Rhodophyceae are widely used as raw material for manufacture of agar in different parts of the world (Table 1). Table 2 mentions the list of red seaweeds used as raw material for agar production in different countries, including India.

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* Revised and updated

Table 1 – Systematic list of red seaweeds used as agarophytes

Class	Order	Family	Genera
Rhodophyta Florideophyceae	Gelidiales	Gelidiaceae	*Gelidium* *Pterocladia*
		Gelidiallaceae	*Gelidiella*
	Cryptonemiales	Endocladiaceae	*Geloipeltis* *Acanthopeltis*
	Gigartinales	Phyllophoraceae	*Ahnfeltia* *Gymnogongrus* *Phyllophora*
		Gracilariaceae	*Gracilaria*
		Hypneaceae	*Hypnea*
		Furcellariaceae	*Furcellaria*
		Solieriaceae	*Eucheuma*
		Gigartinaceae	*Gigartina* *Chondrus* *Iridea* *Suhria*
		Rissocellaceae	*Rissocella*
	Ceramiales	Ceramiaceae	*Ceramium*
		Rhodomelaceae	*Digenia*

The resources of agarophytes in different countries are given in Table 3 and those for India in Table 4. Table 5 gives the names of the countries producing agar for world market and their values.

World Agar Production

John Cappen of the Overseas Development Natural Resources Institute (ODNRI), U.K. put the world agar production around 7000 to 10,000 tonnes annually. Half of it is produced from *Gracilaria* and of this about 3,700 tonnes enter international market. Japan is the top producer and consumer of agar, but depends heavily upon imported *Gracilaria*. In South East Asian region, Thailand, Malaysia and Indonesia are major importers of agar. The developed countries that first manufactured seaweed extracts still produce the bulk of world's supply of this product. Some quantities are also produced in developing countries. The largest single agar production unit is in Chile. Another large factory is under construction in Philippines. Table 6 gives the list of countries producing agar with their capacities.

Table 2 – List of red seaweeds used as agarophytes in different countries

Acanthopeltis japonica Okam	Japan
Aeodea orlitosa (Suhr.) Schm.	S. Africa
Agardhiella tenera (Ag.J.)Schm.	Canada, Pakistan, Brazil, Mexico
Ahnfeltia plicate(Hunds.) Fries	Japan, Russia, UK
Campylaephora hypnoides J. Ag.	Japan
Ceramium bowdenii E.S. Opp.	Japan
Chondrus crispus (L.) Stack.	France., Canada, UK, Ireland, Japan, Pacific, North America
Ceranium rubrum (Hunds) Ag.	Japan
Digenia simplex (Wulf.) Ag.	USA Puerto Rico
Endocladia muricata (Post et. Rupr. Ag. J)	USA
Eucheuma gelatinosum (Esp)J.Ag.	Indonesia
Eucheuma puricatum(Gmel.)Coll	Java, Macassar
Eucheuma denticulatum(Gmel.)W.Bone	E.Asia
Eucheuma serra J.Ag.	E.Asia
Eucheuma striatum Schm.	Tanzania
Furcollaria fastigiata (Huds.) Lam.	Denmark, Canada, Russia
Gelidium amansii Lamx	Japan
G.arboreans Gard	USA
G.cartilagineum (L.)Geillon	Japan, USA, S.Africa
G.caulecantheum J.Ag.	Mexico, New Zealand
G.cornecum(Hids.) Lam.	Japan, S.Africa, Spain, UK
G.crenale(Turn.)Lam.	Japan
G.densium Gard	USA
G.japonicum(Harv.) Okam	Japan, Taiwan
G.latifolium(Grev.)Born	Ireland
G.micropterum Knitz(*Gelidium pusillum*)	India
G.nudifons Gard	USA
G.pristoides(Turn.)Knitz	S.Africa
G.pacificum Okam	Japan, Taiwan
G.pulchellum(Turn.)Knitz	Ireland, UK, USA
G.sesquipedale(Turn.)Thar.	France
G.spinulosum J.Ag.	France

(Table 2 — contd.)

Table 2 – *Contd.*

G.cubcostatum Okam	Taiwan, Japan
Gelidiella acerosa (Feld.) et Hamel	India, Puerto Rico, Japan
Gigartina acicularis(Wulf.)Lam.	S.Africa
Gigartina canaliculata Harv.	USA
Gigartina corymbifera(Knitz.)J. Ag.	USA
Gigartina mamillosa Good et Wooden	France
G.rodula (Esp.) J.Ag.	S.Africa
G.stellata Balt.	UK, Canada, USA
Gloiopeltis furcata(Poster Ruppr.) Ag.J.	USA, Puerto Rico
Gracilaria blodgettii Harv.	Indochina,Japan,N.America
Gracilaria cervicornis (Turb.) J.AG.	USA, Puerto Rico
Gracilaria vernucosa (Huds.) Papenf.	Australia, Canada, Ceylon, China, Taiwan, India, Puerto Rico, Indo Chinan, Japan, New Zealand, S. Africa, USA.
G.corticata J.Ag.	India, Puerto Rico
G.foliifera(Forsk.)Boerg.	Japan, India, USA, Brazil, Puerto Rico
G edulis(Gmel.) Silva	Ceylon, India, Indochina
Gymnogongrus pratens J.Ag.	France
Gymnogongrus jopanica Subr.	Malaya, Indochina
Gracilaria tanax	China
Gracilaria spinosa	China
Hypnea musciformis (Wulf.)Lam.	Philippines, Senegal, Australia, USA, Hawaii, Puerto Rico, Brazil, Mexico
Hypnea specifera (Subr.)Harv	S. Africa
Iridea splendeur (Setch.)Gard.	S. Africa, North America
Phyllophora nervosa	Russia
Pterocladia capillaceae(Gmel.) et Rhur.	Taiwan, Spain, Japan, USA, Australia, New Zealand, UK
Pterocladia pyramidale Gard.	USA
Pterocladia lucida Dawson	New Zealand
P.tanuis Okam	Japan
Rissoella verruculosa(Bert.)Ag.	France
Suhria Vittata J.Ag.	S.Africa

Table 3 – Resources of agarophytes in different countries (Michanek, 1975)

Country	Year	Agarophyte resources in tonnes/year (fresh)
Argentina	1974	21,400
Australia	1970	2,000
Brazil	1973	14,400
Canada	1970	30,000
	1972	1,650
Chile	1970	1,30,000
China	-	500
Cuba	-	2,000
Denmark	1960	30,360
	1964	16,000
Egypt	-	2,000
France	1970	14,000
Indonesia	1971	2,300
India	1991	5,630
Italy	-	20,000
Japan	1971	6,447
Mexico	1974	18,650
Madagascar	-	300
New Zealand	1972	126
Nova Scota	1967	1,500
	1970	9,000
	1973	02,000
	1970	318
Poland	-	200
Republic of Korea	1971	1,800
Spain	1970	8,900
Portugal	1970	23,000
South Africa	-	15,000
Sri Lanka	1961	250
Sudan	-	1,000
Senegal	-	2,000
Tanzania	-	3,000
USA	1966	5,000
	1968	3,303
US Atlantic Coast	1968	100
	1975	50,00,000
USSR	1974	1,76,000
	1975	29,000

Table 4 – Resources of agarophytes in India

Locality	Author(s)	Year	Resources tonnes/year (fresh)
Gulf of Mannar	Varma & Rao	1964	355
Gulf of Kutch	Desai	1967	25
Gulf of Mannar	Umamaheswara Rao	1969	66
Drift Seaweeds at East, West and South East Coasts	Krishnamurthy	1968	53
Tamil Nadu	Subbaramaiah *et al.*	1979	1884
Lakshadweep	Subbaramaiah *et al.*	1979	1516
Tamil Nadu	Csmcri & Cmfri	1986-93	5630

Table 5 – World production of Seaweeds, seaweed gums manufactured therefrom (1980) and their values

Quantity (Q) · tonnes
Value (V) . $ million

Product	Seaweeds		Seaweed gums	
	Q	V	Q	V
Agar	28,000	25	7,000	117
Alginate - a	80,000	20	22,000	125
Carrageenan-b	40,000	16	10,000	55
Total	1,48,000	61	39,000	297

World production of seaweed gums amounts to value about US $ 300 millions of which agar is $ 117 millions, alginates $ 125 millions, carrageenan and furcellaran $ 75 millions. The share of developing countries in the production of agar and world trade in these products remains relatively small.

The red seaweeds used in the production of agar thrive mainly in developing countries, their total value amounts to nearly US $ 45 millions annually i.e., 69% of the world trade in seaweeds. The share of developing countries in production of gums is high and amounts to 90% seaweeds and 38% of agar on the world trade Japan is however, by far the largest known producer of agar; followed by Korea, Spain and Portugal. In all about 200 plants all over the world produce about 7000 tonnes of agar.

Table 6 — Seaweed gums: Estimated production capacities of various countries, 1980

Country	Quantity (Q) : Tonne Agar Q	Number of factories
Argentina	150	1
Brazil	120	2
Chile	387	1
France	80	1
India	90	(44) (1994)
Japan	2,063	170
Rep. of Korea	1,341	14
Mexico	200	2
Morocco	300	2
New Zealand	70	2
Portugal	710	4
South Africa	070	1
Spain	1,200	3
United States	200	1

Source. ITC estimates based on recorded imports and exports, various reports and discussion with industry respresentatives.

The Market for Agar

ITC Geneva estimated world production of agar over 7000 tonnes/annum. Japan produces 2000 tonnes/year, followed by Republic of Korea 1,300 tonnes/annum, Spain 1200 tonnes/annum and Portugal 700 tonnes/annum. Developing countries account for some 40% of world production, the production distribution being: Asia 58%, Latin America 31% and Africa 11% and taking developed and developing countries together, world production of agar is as follows: Asia 50%, Europe 30%, North and South America 15% and Africa 5%. Most of agar is produced from *Gracilaria*, but about 1000 tonnes per year of bacteriological grade agar is made mainly from *Gelidium* and *Pterocladia*.

Japan : is the worlds largest user of agar and like the Asian region in general, it consumes almost as much agar as it produces. It produces about 2000 tonnes/annum of agar. The main seaweeds used in the production of agar are: *Gelidium, Gracilaria, Acanthopeltis* and *Pterocladia*. The structure of agar industry however,

changed. The small Japanese production units work mainly with *Gelidium* spp. and with varying amounts of *Gracilaria* and *Ceramium* spp.

About 17 factories in the country extract agar by mechanical freezing and prodution reached 1,099 tonnes in 1979. 70% of agar in Japan is produced from *Gelidium, Aconthopeltis* and *Pterocladia*. Although it produces seaweeds, it also imports them in large quantities. In addition to this the raw agar is imported from Chile, Argentina, and South Africa. *Gelidium* harvests (from three species) are *G.amansii* 3,100 tonnes/annum, *G.subcostatum* 620 tonnes/annum and *G.japonicum* 330 tonnes/annum.

Canada : Nova Scotia and Gulf of Lawrence Coasts of Canada are some of the richest seaweed areas in the world. For Nova Scotia fishermen seaweed harvesting is an industry worth more than US $ 1 millions. Half a dozen seaweed corporations are now buying from Nova Scotia. Four drying plants are located in the province. During 1967-70, 95423 tonnes of agarophytes were collected fetching a value of US $ 4.3 millions (Michanek, 1975). The "Irish Moss". *Chondrus crispus*, industry in this country which was started as an adjunct endeavour to the lobster fishery, has increased considerably in the second half of 1960s. The Prince Edward Island harvest of *Chondrus* (agarophyte in Canada) is the largest in the North-West Atlantic. In 1970 it accounted for 30,000 tonnes (value US $ 6 millions). An estimated 11,500-13, 500 tonnes (fresh weight) of "Irish Moss" was harvested in Prince Edward Island (Morrison, 1973). Canada remains major source of *Chondrus* (agarophyte in Canada). The harvests of *Chondrus* in Nova Scotia and Prince Edward Island peaked at over 12,000 tonnes/year in the early 1970s when 11 drying factories were in operation. It declined to around 7,000 tonnes in 1979, owing to the dramatic increase in the production of *Eucheuma* in the Philippines.

USA : From the New England down to Bostan there is a rich rocky shore with marine flora A harvestable quantity of 5,000 to 9,000 tonnes (wet weight) of *Chondrus* was estimated at Maime (French, 1970).

Marine Colloids Inc., probably the worlds largest producer of extracts, has a carrageenan plant at Rockland, Maime. This state has eight carrageenan factories; Krafts Food at Portland. Raw material is also imported frcm Canada. The total harvest of *Chondrus* in the United States (North-West Atlantic) amounted to 3,300 tonnes in 1968 In Barnegat Bay, New Jersey (Moeller, 1964) 3 tonnes of *Gracilaria* and 5 tonnes of *Agardhuella* were estimated. The approximate value of the total standing crop of *Gracilaria verrucosa* covered in 44 km^2 of the total 120 km^2 of the bay would be US $ 3,50,000. The high cost of labour in the United States discourages any extensive harvesting of seaweeds for American Agar and Chemical Company, which is one of the main producer of agar in the United States. There are reasons to believe that Southern California, if thoroughly but sensibly exploited could yield more than 1,500 tonnes of fresh agarophyte per annum (Tseng, 1955). *Gelidium* grows abundantly from point Conception of Magdeleno Bay in Baja California. Commercial production of agar in the United States has undergone

several ups and down. In early eighties only one firm, American Agar and Chemical Company of San Diego was in operation. This firm produced high grade beacteriological agar using *Gelidium* from Maxico and many other countries. USA Agar Company, National City, California is the nucleus of the present American Agar Co. Its production in 1942 was 4,000 km. In 1945, eight factories existed.

Now 200-300 tonnes of agar per annum is being produced. The local weed is not sufficient to meet the full need of the American Agar Co. and it is now bought from South America, South Africa, Portugal and N. Africa. In Baja, california eight companies have earmarked certain areas along the coast for harvesting, the most important being Agar-mex. South America with 27 areas and Gel-Mex with 35 areas.

The United States seaweed industry is the largest in the world. Some of its producers are transnational companies. About 200 tonnes/annum of high grade bacteriological agar is produced by American Agar Chemicals, Co. It imports seaweed supplies from Chile and Mexio. Its annual demand for agar is 1,000 tonnes and is a major importer of Agar.

Marine Colloids manufactures all of them in the United States. Domestic supply of carrageenana, is estimated at 5,000 tonnes yearly. The company imports most of its seaweed supply from developing countries. In Phillippines, where it has collaborated in the development of seaweed resources, it also undertakes preliminary Processing.

Marine Colloids was formed in 1959 from an amalgamation of Algin Corporation of America and Seaplant Chemical Corporation.

Mexico : Mexico seaweed processing industry is located in the state of Baja California. It produces some 200 tonnes/year of agar from *Gelidium robustum*. Production includes about 25 tonnes/year of high quality bacteriological agar, most of which is exported to the United States. The other types of agar produced are mainly consumed in the country.

Mexico produces about 1,000 to 2,000 tonnes annually of *Gelidium* which it uses mainly in the production of agar. Mexico may develop agar industry as a priority. The country is endowed with the technical and financial resources required to exploit this potential. Its current pace of economic and industrial activity is another favourable factor.

Denmark : In addition to the processing of indigenous resources by the Furcellaran Industry, there is also a Danish phycocolloid Industry working up imported material, viz., *Chondrus crispus* from the Canadian Maritimes. *Eucheuma* and other agaroid materials are imported from the Far East and smaller quantities from South Africa and S. America. Approximately 16,000 tonnes of agar weeds were harvested annually (Druehl, 1972) producing 830 tonnes of agar approximately.

Denmark produces 3,400 tonnes/year of carrageenan and 1,000 tonnes/year of "Furcellaran". Litex Industries started producing "Danish Agar" now as furcellaran

and carrageenan and its total production is 2,200 tonnes of which 1,200 tonnes is carrageenan.

Chile : Kim (1970) estimated the standing crop of 11 species of *Gracilaria* totalling to 1,30,000 tonnes. In 1968 production was 1,600 tonnes while a total of 374 tonnes of dry material of *Iridea, Gelidium* and *Gigartina* was exported. One factory in Chile manufactures agar with a capacity of 300 tonnes of agar per year (Michanek, 1975).

The country has the world's largest factory for agar prodction which was set up with foreign collaboration. The factory exports most of its production of about 380 tonnes/annum and it handles the balance to the domestic market consumption, whose requirement is around 50 tonnes/annum. The total annual turn over of the seaweed processing industry is estimated at US $ 3 millions. Chile exports agar to fairly large number of countries.

Australia : *Gracilaria verrucosa* is the principal agarophyte. In bay area of South Sydeney 3 men in a trawler collected 270-300 kg/hour (fresh) seaweed (Chapman, 1970). Along the 1,370 km coastline of New South Wales it has been estimated that there is enough seaweed supply for the manufacture of 1,000 tonnes of agar/year. Kara Vitta Company produces 1 tonne of agar from 7 tonnes of seaweed. 20 men collect and bleach 8 tonnes of wet weed/day. Western Australia is still manufacturing agar like gelan from *Eucheumaspeciosum* and *Hypnea musciformis* (Chapman, 1970).

New Zealand : *Pterocladia* is used as raw material for agar manufacture. Kirby (1957) reviewed processing, properties and economics of seaweed in detail. Schwarz (1953) gave an account of seaweed utilization in New Zealand with an emphasis on manufacturing problems. The seaweed is sun dried, packed and sent to factory in South Island. Watkinson and Smith (1972) gave harvests of *Pterocladia* at 126 t/year (dry) on an average. The sole manufacturing firm is Davis Gelatin Co. of Christchurch. Single collector in a rich area collects about 50 lbs. (*Pterocladia*) per tide (Chapman, 1970).

The New Zealand agar industry was established by Davis Gelatin (NZ) Ltd about 40 years ago to meet escalating demand during Second World War. The industry currently produces high-quality bacteriological grade agar for coming industry. It uses the seaweeds *Pterocladia pinnata* and the larger *P. lucida*. A second agar factory, Coast Biological Ltd., has recently been established at Opotiki North Island, which may increase domestic production to well over 100 tonnes/annum.

Norway : In 1970 Norway utilized 79,500 tonnes of seaweeds including *Gigartina* and in the previous years the amount utilized was between 55,000 and 8,57,000 tonnes.

The Netherlands : Chapman (1970) quoting from Spoon (1951) mentioned that two factories existed producing agar from *Eucheuma gelatinosa* and *Gloiopeltis coliformis*.

United Kingdom : Ireland - "Irish Moss" - *Chondrus crispus* is the main agarophyte. In one of the war years, 600 tonnes was collected and agar has also been manufactured from *Gelidium pulchellum* and *G. latifolium* and since then these two species which occur in sufficient quantities, are suitable substitutes for *Chondrus* and *Gigartina*. Production of agar commenced in 1943 - 10 c. wt. was manufactured. It was 44 tonnes in 1944. At present there is no production.

The country imports over 300 tonnes/year of agar and about 450 tonnes/year of carrageenan to meet the domestic consumption.

France : 4,400 tonnes of wet *Chondrus* and *Gigartina* agaroid was produced during 1950-51. According to French figures submitted to FAO Year Book of Fisheries Statistics, 1883 tonnes and 3,192t of *Chondrus crispus* were available in 1965 and 1970 respectively. The price/kg in 1970 was 0.43 French France (Michanek, 1975).

Sobigel S.A. produces about 60-80 tonnes of agar yearly from *Gracilaria*. In 1978, France exported 33 tonnes of agar of EEC and imported 53 tonnes. Taking domestic production into consideration the domestic market for agar may be around 10 tonnes/year. France first produced Carrageenan in 1911. Todays largest manufacturer is Satia, a subsidiary of Ceca, A.A. It produces 2,500 tonnes/year of carrageenan and is the world's second largest producer of the gum. The company became part of the Pierrefitte-Auby group. A second company IRANE S.A. produces some carrageenan for Pierrefitte-Auby group.

Spain : The Spanish seaweed industry is mainly engaged in the production of agar from *Gelidium* and is the second largest producer in the world, exceeded only by Japan. Local consumption is small and Spain is a leading exporter of agar in the world market (Spain, SNIQ, 1968). In 1970, 8,900 tonnes of the raw *Gelidium* was harvested. According to the figures available for the last few years, 1.000 tonnes of the "Carrageenan" - *Gigartina* and *Chondrus* were harvested. Various industrial aspects of seaweed utilization have been treated in Spain. in SNIQ (1968). 60-70% of *Gelidium* is picked upon the beaches which casts ashore during stormy and rainy season. Consequently a number of seaweed processing factories are located around Madrid and Burgos. Regional requirements have alone been considered when two new carrageenan factories with joint rated capacity of 580 tonnes/year were built in the "development" area of Vigo and Burgos. From May to October, at a low tide, some *Gelidium* and 70-80% of 'Liquen' (*Chondrus* and *Gigartina*) is torn off the rocks and dried in the sun. Superior quality of *Gelidium* is collected by Frogmen during these months; 100 small ships are operating with a skipper, a mechanic and four divers 15-20% of *Gelidium* is harvested in this manner.

Three companies i.e., Prona.S.A., Gomas Marina and Novagel S.A. currently produce agar about 1,000 tonnes/annum. Processing started some 40 years ago using local supplies of *Gelidium* and *Gracilaria*. The country first produced carrageenan in 1970. Two producers Hispan agar S.A. and CEAMSA have total capacity about 500 tonnes/year. Under normal conditions local seaweed supplies

are adequate and occasionally it imports *Gracilaria* which amounted 432 tonnes from Chile in 1978.

Portugal : There are six factories with a total capacity of 1620 tonnes of agar. Four of them exclusively use *Gelidium sesquipedale* while the other two employ primarily *Pterocladia capillacea, Gelidium attentatum. G.latifolium* and *G.pusillum*. The actual quantity was 21,000 tonnes (fresh) in 1970. The estimates (dry weight) of agarophytes on the mainland and Azores are 6,000 and 1,200 tonnes respectively and the carrageenophytes *Chondrus crispus, Gigartina stellata* and *G.acicularia* are approximately 1,200 tonnes. The predicted output is 1,300 tonnes of agar and 4,000 tonnes of carrageenan (Palminha, 1971). Much of the red algae is collected in the tidal area at low tide, and outside this divers work from small boats. On the mainland and in the Azores 114 boats and 670 divers operate. Collection of seaweeds fixed to the substrate is regulated by low restricted to certain period of year.

Portugal produces about 700 tonnes/annum of agar and 200 tonnes of carrageenan. Two companies Sicomol and Iber agar manufacture agar. Sicomol also produces bacteriological grade agar. Most agar is made from locally obtained *Gelidium* Spp. and about 1,100 tonnes/year are said to be available. Some agar is made from *Gracilaria*. Trade sources report that about 350-400 tonnes/year of *Chondrus* are collected from which Iber agar produces about 100 tonnes/year of carrageenan. It appears that seaweed supply is adequate for domestic demand. Iber agar has factory in the Azores which produces about 150 tonnes/year of agar. An Italian company-Ala, also in the Azores, produces around 100 tonnes/year of this seaweed gum. Both factories use *Pterocladia pinnata* producing high quality product. Nearly 50% of the agar and carrageenan produced are exported to the United States, the other important export markets are EEC, Japan. Exports exceed US $ 1.3 million annually. Recent studies of the beds of *Pterocladia pinnata* in the Azores have shown about 2000 tonnes/annum.

Morocco : Morocco is one of the greatest producers of agar followed by Japan, Korea, Spain and Portugal (Michanek, 1975). Approximately 3,000 tonnes/year of *Gelidium sesquipedale*, the main agarophyte is harvested. At present there are four factories in Casoblance, Berrachied, Tanger and Kanitra, which produce agar. The latter has used Japanese techniques and has a capacity of 1,000 tonnes/year (de Craene, 1971). According to Rodriguez (1953) the yield of *Gelidium* from the shores of the Canary Archepelogo can be reckoned at approximately 100 tonnes/year. The agarophyte used is *Gracilaria.*

Morocco's production of agar is estimated at about 300 tonnes/year most of which is exported to EEC, the United States and Japan. Exports are valued at about US $ 3 million per annum. Since 1974/75 agar production has been controlled by Marak agar, S.A., a company formed from the merger of four companies. Current collection of Gelidium is geared to the requirement of the domestic processing

industry. The country has good prospects for immediate development for agar industry.

Brazil : In North Brazil an extensive seaweed utilization is being launched. The Algimar Company has built three factories in the states of RIO grande do Norte, Ceara and Paraíba and organised 165 collection centres over about 2,000 km giving work to 2,40,000 people with strong support from Brazilian Government. The main agarophytes harvested are *Hypnea musciformis* and *Gracilaria* and the minor one being *Glidiella*, *Gelidium* and *Pterocladia*. Agar production capacity is 500 tonnes/year. 1973 production figures for agar are 144 tonnes/year from a harvest of 14,400 tonnes of agarophytes. The production is correlated to the development of stock raising in the inner parts of the country.

A plant in Sao Paulo has been producing agar since 1973. about 120 tonnes/annum is being produced but some quantities of agar are imported to meet domestic demand. Brazilian production of *Gracilaria* reached peak in 1979 with about 2,000 tonnes dry agar yearly exported to Japan. Brazil has the potential for increasing agar production and processing of it agar on the way.

Argentina : The seaweed production increased from 2,000 tonnes in 1958 to 24,800 tonnes in 1968. Red algae harvested in 1973 was 21,000 tonnes. Exports of Marine algae and agar-agar amounted to US $ 1.1 million in 1669. The important agarophytes are *Gracilaria* and *Gigartina*.

A plant set up in the Argentinian Province of Chubut currently produces agar about 150 tonnes per year and also produces small quantities of carrageenan from *Eucheuma* which it imports mainly from Philippines. The major market for both agar and carrageenan is most probably the food sector, which uses them mainly in milk products. A new plant was set up in 1983 to enable Argentina to seek regular export market. *Gracilaria* collected locally meets the demand and also export market. About 1,200 tonnes were exported.

Peru : Peru imports small quantities of various seaweed products including carrageenan the demand for which is at 40 tonnes/year. Peru exported 700 tonnes *Gigartina* and 550 tonnes *Gracilaria* the most important seaweed. The Country is known to have substantial seaweed resources and there are many changes for reviving production of agar as also the agar-bearing *Gracilaria*.

China : Agar was originally produced in China and was later introduced into Japan in 1662. The industry declined, however, in 1937. China has only three factories producing about 35 tonnes of agar and production still continues. *Gelidium divericatum* and *Gracilaria verrucosa* are the main agarophytes. *Gloiopeltis furcata* is used for obtaining a paste employed in sizing of fabrics. Over 500 t of this alga is being harvested per year.

With its annual production of over 2,75,000 tonnes of dry weight, China is by far the world's largest producer of seaweeds.

Agar production entirely in 'Taiwan' Province estimated at 150 tonnes/year. Species of *Gracilaria* are mainly used. And the cultivation method for this seaweed introduced in the early 1960s now produces 12,000 tonnes/year. About 11,000 tonnes are used as feed stuff in the culture of abalone. About 300-400 tonnes/year of the alkali-treated seaweeds are exported to Japan at over US $ 2,000 per ton at roughly twice the price of unprocessed dried seaweed, the balance is sold to local seaweed gum industry.

Republic of Korea : This is probably the world's third largest producer of the seaweeds with regard to quantity and second largest with regard to value (Michanek, 1975). About 35.8 thousand tonnes of red algae were harvested in 1971 (FAO, 1971). 1,800 tonnes (Wet) of *Gelidium amansii* and other *Gelidium species* were collected annually. Boney (1965) quotes Thivy's 1952 estimates of the total standing crop of agarophytes of Korea at 1,103 tonnes (fresh).

This country produces about 1,350 tonnes/year of agar and over 100 tonnes/year carrageenan and has well established export trade in these products. Fourteen factories manufacture agar from the seaweed *Gelidium amansii*. Nearly 90% of production is strip agar, the balance is in powder form. The country exports nearly 50% of its agar mainly to other Asia region. In 1979 these exports exceeded to US $ 6 million. Japan imports over 300 tonnes/year from this source, the other importers are Hong Kong, Thailand and Malaysia Production of the most important seaweed *G amansii* is estimated at 7,000 tonnes annually. According to the Korean Institute of Science & Technology, production by mariculture of *G. amansii* amounts to 60 tonnes/year.

Agar production is expected to be increased because of regular supply of seaweeds available from Philippines.

Indonesia : In 1971, 2,300 tonnes of wet seaweeds were harvested. The principal agarophytes are *Gracilaria, Gelidium, Hypnea* and *Eucheuma.*

Philippines : *Eucheuma* is commercially most important seaweed as carrageenan source and has been exported to Japan and USA. Its commercial cultivation is one of the greatest sources of income. In 1973 Filippimo farmers established 86 *Eucheuma* farms containing over half a million People. It is estimated that cultivated source will account for more than half of the Philippine commercial production in 1975. Doty (1973) estimated 30 tonnes (fresh) of *Eucheuma* per hectare on a small scale farm. The other agrophyte *Gracilaria* is harvested @ 35 kg/day

The Philippine seaweed harvest dwindled from 1,000 tonnes in 1966 to 268 t in 1970, the exports from 805 tonnes (dry) in 1966 to 264 tonnes (dry) in 1968 of which 229 tonnes went to USA. In 1972 the exports were 570 tonnes (dry).

The Philippines have recently started the preliminary processing of about 600 tonnes/year of *Eucheuma* for carrageenan production. *E.cottonii* makes up the bulk of export of *Eucheuma*. With the introduction of mariculture, the Philippines over the last eight years has increased production of *Eucheuma* from less than

100 tonnes/year to an annual average of 13,5000 tonnes valued at US $ 7 millions (1979-90). As a result the country has become the largest supplier of this seaweed. The experience it has gained in the cultivation of *Eucheuma* should help Philippines to increase production of other seaweeds. The countries potential, particularly in the production of seaweeds is encouraging.

Singapore : Singapore produces neither seaweed gums nor seaweeds but it is an important trading centre for the seaweeds that grow in the neighbouring countries. Some major producers of carrageenan are reported to have offices in the country. Its importance as a trading centre for seaweed gums is likely to grow with the developing seaweed industry in the region.

History of Agar in India

Agar was first produced commercially in Japan in 1670. During Second World War, agar was classified as "critical war material". Consequently following countries started producing it in substantial quantities : Britain, Denmark, Australia, United States, Russia, New Zealand, South Africa, Brazil, Canada, Spain, Italy and many other countries. This impact was also felt in 1940s in India. In 1942, the Council of Scientific & Industrial Research arranged manufacture of agar for the first time at Research Department of the University of Travancore and during the period 1942-46 a small supply was maintained, chiefly for preparation of cholera vaccine. *Gracilaria* was used as raw material. Later, attempts were made to assess seaweed resources (cf Desikachary, 1967 and Krishnamurthy, 1971 for detailed information). Fifty's and Sixty's saw new interest in this field. A definite concerted effort to produce agar on pilot-scale was made (Kappanna & Rao, 1963 and Rao *et al.*, 1965) at the Central Salt and Marine Chemicals Research Institute, Bhavnagar. Simultaneously some efforts were made at Central Marine Fisheries Research Institute (Thivy, 1960) and by certain State Government Fisheries Departments. This encouraged many foreign firms to get interested in Indian agarophytes. Thus, India became a leading exporter of seaweeds, especially agarophytes. And during the period 1966-76 about 200 tonnes of dry agarophytes were exported annualy (Krishnamurthy, 1971). Even though the export of seaweeds of all kinds was banned completely till 1970 as per the Export Trade Control order, the export figures of seaweeds including agarophytes have picked up in 1980's and export figures are:

Year	1989	1990	1991	1992
Qty (kg.) dry	107895	100	-	15260
Value (Rs.)	1688767	27455	-	352765

The agar industry in India uses mainly *Gracilaria* and *Gelidiella* as raw materials. These seaweeds are at present collected from the South east cost of Tamil Nadu from Rameshwaram to Kanyakumari and it is an important source of income

for fishermen in many of the costal villages of Ramanathapuram (Ramnad) district in Tamil Nadu. The highest price is usually paid for *Gelidiela* Rs.8,000/tonne and *Gracilaria* Rs.3,000/tonne (dry seaweed). The seaweed collection has now become an organised business involving seaweed suppliers who sell the sea weeds to the manufacturers of agar. They employ seaweed collectors who are paid remunerative rates which varies with the type of seaweed collected. Presently, mostly fishermen (including women) are being engaged in collection of seaweeds.

For the most part, Indian agar factories are located in Tamil Nadu, close to the ''seaweed'' belts. There are a few factories in Kerala and Andhra Pradesh whose out put range from 10-60 kg/day. The newest factories plan to produce upto 100 kg/day. And at present about many firms are manufacturing agar in medium/small/cottage scale units. Table 7 gives the list of agar companies in operation at present. The other statistics like cost of production, rated capacity and present production figures are not relevant in the present context of market trend which always fluctuate in the case of agar. However, Cellulose products of India, Madurai, still remains the leading manufacturer of agar with a present production capacity of 20-25 tonnes/annum and the selling price of agar per kg being Rs.250-350. The present over all market rate of agar varies between Rs.150-400.

Two grades of agar are manufactured. Food grade, which is usually produced in mat form and IP grade which conforms to Indian Pharmacopoea and is usually sold as powder. Other types of agar are also sold in strip mesh and granule forms. For food paleness of colour is usually deemed more important than gel strength and whereas for industrial and other purpose gel strength is measure of consumption. *Gracilaria* which is cheaper and easy to bleach or *Gracilaria/Gelidiella* mixtures are the usual raw material for production of food grade agar. When high gel strength is required *Gelidiella* alone is used to produce food grade agar. IP grade agar is also produced from *Gelidiella.*

India produces about 90 tonnes of agar-agar and is expected to go up to 140 tonnes over the next few years. And the main hurdle in the way of increased production of agar in India is severe scarcity for raw material *Gracilaria* and *Gelidiella.*

The value of agar produced at full rated capacity would be Rs.25-30 crores annually. However this cannot be achieved due to several technical problems. Inspite of all these, agar is being exported and one firm had exported 5 tonnes of agar during 1970-72. And the latest export figures for agar is about 22 tonnes per annum and are met from the domestic production. Several countries have been purchasing Indian agar.

Agarophyte Resources in India

The resources of agarophytes in India are given Table 4. Although many workers have given different estimates from 1964 to 1979, of the standing crops of agarophytes, the most recent and scientifically and intensively conducted sublit-

Table 7 – Production of agar in india at present

Company Name	Production Kg/day
Carbose India	15
Omega Marine Chemicals	10
Subbalakshmi Agar Agar Industries	12
Nellai Agar Agar Industries	20
Freinds Chemicals	5
Kanya Marine Products	10
Alfa Agars	5
Madirai Agar Industries	10
Marine Gel	12
Sri Vignesh Agars	15
Preethi Agar Agar Industries	5
Reshmi Agar Industries	15
Sri Industries	20
Shri Chem	20
Micro Chem	20
Madurai Agars	15
Jayan Industries	15
Gunasekaran	5
Mayee	10
Vinayaga Agars	15
Balaji Agars	5
Anu Chemicals	12
Kavitha Agar Industries	15
Bismi Agars	10
Utharam	5
Cellulose Products of India Ltd.	50
Srivas Chemical	50
Quixgel Biochemicals	50
Virable Agar Agar	Production figures not available
Madurai Agar & Chemicals	10
Marine Chemicals	50
SNAP Natural and Alginate Products	Production figures not available
Kothari Phytochemicals International	-do-
Devi Bio-chemicals	10
Seaplant Chemicals	10
Maruthi Agar & Co	10
Seaweeds of India	5
Hindustan Agar Co	5
Gel Enterprises	10
Gel Products (Pvt) Ltd.	5
Gurukripa Chemicals	5
Uma Polymers (Pvt) Ltd.	50

Also South Sea Chemicals, Indo Nippan Sea Fords, Aswini Agar Co., East coast Agar Produce agar but their production figures are not available.

toral survey of seaweeds of South India the Chief supply centre of sea weeds to industry in the country) and Lakshadweep Islands in the Arabian Sea, reveals that the total standing crop of all agarophytes *Gelidiella*, *Gracilaria* and *Hypnea* put together is about 2,860 tonnes (fresh). The bulk of this comprises *Gracilaria* and *Hypnea* totalling to about 2,195 tonnes (fresh) only (Subbaramaiah *et al.*, 1979, a,b). The latest resources of agarophytes obtained from deep waters upto 20m depth survey in Tamil Nadu conducted jointly by CSMCRI & CMFRI during 1986-91 stand at 5630 tonnes (wet)over a productive area 297.5 sq-km. And these resources are completely devoid of *Gelidiella* which is the principal agarophyte. *Gracilaria* accounts for 630 tonnes (excluding *G. edulis* and *G. corticata*) and *Hypnea* spp. account for 5000 tonnes. With the present production capacity about 90/tonnes/annum, three fourths of the rated capacity of 140 tonnes/annum, the available seaweeds may not sustain the agar industry for ever. If the *Gelidiella* resources are alone taken for consumption, the industry cannot sustain any longer, notwithstanding the fact of meager resources of other agarophytes.

Processing of Raw Materials

Although the process of extraction of agar the world over involves similar steps, it may vary slightly with the type of material employed and location of the plant. In India, the process mainly involves the following steps as developed by Rao *et al.*, (1965): (1) cleaning and washing of seaweed; (2) Removal of impurities; (3) Chemical treatment for bleaching; (6) Gelation and preparation for freezing; (7) Freezing-separation of agar from remaining impurities frozen in ice; (8) Washing-removal of impurities — Thawing; (9) Removal of colour and sterilizing and washing; (10) Drying; (11) Pulverizing and packing.

The agar thus obtained contains; moisture 15-18%, ash 4%, nitrogen 0.2-0.3%. It has a gel strength of 180-300 g/sq cm. The gel melts at 83°-90°C and sets at 37°-39°C, thus conforming to USP.

Japanese Process

At present there are about 500 plants which are manufacturing agar as per following methods (Okazaki, 1971):

(1)	Industrial Refrigeration	(8)	Cutting
(2)	Mixing	(9)	Freezing in cans
(3)	Washing	(10)	Refrigeration at –10° to 20°C for 30 hrs
(4)	Boiling in pressure cooker	(11)	Thawing
(5)	Primary filtration	(12)	Washing
(6)	Terminal filtration	(13)	Drying
(7)	Coagulation in pipe	(14)	Packing

The average yield of agar by this process is 25-29%. Examination and grading of agar is carried out by the Japanese Agar Distribution Control Co. If there is more than 3% crude protein it is rejected for export. Specifications for Export Grades are as under.

	Solidity	% Crude protein	% Insoluble materials
Ist Grade	300	1.5	2
2nd Grade	200	2.0	3
3rd Grade	100	3.0	4

Moisture: 22% by weight

Thus, purified agar contains 90% moisture. This is dried in air unit and 35% moisture is left. It is bleached in 1% sodium hypochlorate at room temperature. Excess bleaching agent is reduced by Na_2SO_3 after which the agar is removed, washed and dried until about 20% moisture remains. (1lb. = 453.6 gm)

USP Grade of Agar as determined in Gelidium Agar (Chapman, 1970)

Moisture	15-18%
Ash	4-6%
Nitrogen	0 2-0.3%
Gel Strength	300g/cm^2
Gel temp.	37-90^{0}C
Melt. temp.	83-90^{0}C
Colour	Near white
Japanese standard	
Moisture	22%
Ash	4%
melting residues	2%
Crude protein	1.5%
Gel strength	350g/cm^2 or more
Colour	White & lustrous

Chemistry of Agar

Agar, formerly for the most part called agar-agar is a gel forming extract from certain Rhodophyceae and the Seaweeds yielding agar being termed as agarophytes.

Tseng (1945) referred to the extracts from Rhodophyceae viz., agar, carrageenan, etc. which form more or less jellies in aqueous solutions as gelose. Staloff (1957) refers to water extractable polysacchrides from Rhodophyceae as polysac-

charide hydrocolloids and divide them into three groups; agars, carrageenans and gelans. Gelans are similar to carrageenans regarding viscocity and gelation preparation, but without the ability to aglomerate ease in and precipitate methyl blue. Furcellaran and Hypnean are grouped with gelans (Levring *et al.*, 1969). A common component of all red algae cannot be chemically described because it varies from species to species, with geographical location and environmental parameters. Generally, agar is extracted with hot water followed by a series of steps to purity as outlined earlier. In some cases the seaweeds are washed alternatively in alkali or electrolytes or acid treatment is given below 25°C, prior to hot water extraction. The resultant agar may be classified according to the following (Druehl, 1972):

Gelidium type	Gels firmly if dilute
Gracilaria & Hypnea type	Gels firmly if concentrated or electrolytes added
Chondrus type	Gels firmly only if very concentrated

More recently Levring *et al.*, (1969) suggested for true agar a gel point of 33°-39°C and melting point of not below 85°C for 1 5% solution of agar.

Chemical studies of agar from *Gelidium amansii* revealed it to be a mixture of two polysaccharides, agarose and agaropectin. Molecules of agarose are composed of neutral chain of β-D-galactopyrenose residue linked through 1,3 positions and 3,6- anhydro L-galactopyrenose connected through 1,4-positions and repeated alternatively (Volesky *et al.*, 1971). Agaropectin has the same linkage as agarose but also contains ester sulphate, glucuronic acid and pyruvic acid (Araki, 1965).

The agar from *Gelididum* and *Gracilaria* is primarily of two components, a neutral polymer (agarose) and a sulphated polysaccharide (agaropectin). The gelling ability was found to be due to agarose (Tsuchiya & Hong, 1965). Ratio of agarose to agaropectin in Agar is as follows:

Expt. No	Materials	Acetylated agarose(%)	Acetylated agaropectin	Agarose/ agaropectin
1.	*Gelidium* agar	50.0	39 5	1.3
2	*Gelidium* agar	56 7	34 4	1.6
3	*Gracilaria* agar	75 7	3 6	21 0
4	*Gracilaria* agar	75.5	3.8	19.9

The ratio of agarose and agaropectin differed greatly in the different varieties of agars. The infrared spectra of agar, agarose and agaropectin for *Gelidium amansii* and *Gracilaria* spp. were almost identical The gel strength is mainly attributed to agarose present in agar. Fractionation of *Gracilaria* agar by Duckworth and Yaphe (1971) showed that there are at least three extremes in structure 1. Essentially natural agarose 2 Pyruvated agarose with sulphation and 3. highly sulphated

galactons. The properties of agar differ from species to species reflecting differences in the properties of these extremes in structure (Young, 1973). The same author had studied in detail the fractionation of agar from *Gracilaria* (1974). 5-8%; in *Hypnea* 10-12% and in *Chondrus* over 16% (Upham, 1967). Greater the percentage of this ester sulphate lesser is the gel strength. Schachat and Raymonds (1965); Humm and Williams (1948); Upham (1967); Rama Rao and Krishnamurthy (1968) have shown that *Hypnea* agar is characteristic in its flexible properties because of the independent controllable properties of gel strength and gelling temperature due to alterable condition of ester sulphate in agar. It has been proved beyond doubt that the gel strength of *Hypnea* agar can be controlled (Schachat & Raymonds, 1968; Rama Rao & Krishnamurthy; 1968 and Upham, 1967), However, addition of optimum concentration of an electrolyte is desired beyond which negative effects crop in.

As early as 1938, Yasagawa showed the effect of alkali (NaOH) on seaweed mucilages of *Gracilaria* and other agars. He considered this alkali treatment to have probably caused the conversion of the algal polysaccharide from low to high setting power in a similar manner to that obtained with *Gelidium amansii* after splitting of large proportions of sulphuric acid from the sulphate ester. And this was confirmed by many later workers (Schachat & Raymonds, 1965; Upham, 1967). Doshi *et al.* (1968) have shown that small dose of gamma radiation enhances the gel strength of agar. The gel strength increases with increase in dosage of radiation upto a critical dose which differs from species to species. The increase in gel strength is attributed to the breaking loose of the bound sulphate ester in the agar molecule.

Use of Agar

Robert Kotch first showed in 1881 that agar was an effective culture medium as many micro-organisms were unable to decompose it. Thus, it has become a standard fixture in most hospitals and laboratories where culture media are required. Agar is also useful for stomach disorders (Mathieson, 1972). It is a non-irritant bulk producer which absorbs and holds water as well as serving as a mild laxative. It is now employed as a coating for various capsules (antibiotics, sulpha compounds and vitamins) when a slow release of the medicant is desired at a point beyond the stomach. Agar preparations are sometimes used beneath bandages to heal wounds. In addition, it has a variety of other uses, preservation of canned meat, fish and other easily spoiled food, as an ingredient of water proof paper and cloth preparation, sizing for fabrics, cleaning medium, a clarifying agent in manufacturing of wines, beers and coffee, and the preparation of special breads for diabetics of which agar replaces the starch. Other large uses are in the bakery and confectionary industries, and in dentistry. It is used in suspending, thickening, stabilizing and gelling aqueous solutions. It also finds a place in the preparation of syrups, ice-creams, jam, salads, desserts, etc. Some of the most important uses and users of agar are given below:

Uses	*Users*
Resilient	Dentists, Criminalists
Matrices	Sculptors
Stabilizer	Meats, sherbets, bakers, confections
Clarifier	Vintners
Starch substitute	Health foods manufacturers
Gelling agent	Meat, paper, confections, jams, jellies etc.
Inert-base	Public health, technologists, orchid growers
Suspending agent	Radiologists, cosmetic manufactures
Emulsifying agent	Manufacturers of Pharamaceuticals, dairy products and chemists
Colloids	Chemists
Sizing medium	Paper and textile manufacturers
Lubricant	Wire drawers
Core binder	Precisions and foundaries

Some of the most important uses in industry are in photography, replacing gelatin for special application, for hyperthin, reduction emulsion, storage cell separators and analytical coagulant of the SO_4, $AsSO_2$, $Fe(NH)_2$ and $BaSO_4$.

Future Prospects of Agar Industry

Demand

Asia consumes about 3,500 tonnes of agar annually and accounts for 50% of the world demand for this seaweed gum. Japan is the world's largest user and like the Asian region in general, it consumes almost as much agar as it produces.

The United States, major market for agar, consumes an estimated agar of about 1200 tonnes annually, which are largely met by imports. Domestic production reaches only 200 tonnes annually. Demand in EEC is less than 1000 tonnes/year. 90% of it is met by imports. UK, Federal Republic of Germany account for 65% of the total demand and production nil, other EEC countries consume less than 100/tonnes/year. France produces 80 tonnes/year for domestic consumption and for export. Export figures of agar from selected countries are given in Table 8.

Demand for bacteriological grade is estimated at 1,000 tonnes/year and comes mainly from the industrialised countries, although demand in developing countries is on the rise.

Demand for agar in the BOBP region (Bay of Bengal Programme) viz., Thailand, Malaysia, India, Bangaladesh, Indonesia, Sri Lanka, Korea is met largely by imports which have been fairly consistant. Over all, the BOBP region imports around 700 tonnes of agar per year i.e., around 20% of the total world production of agar.

Table 8 – Exports of seaweed gums from selected countries, 1980 (tonnes)

Country	Agar	
	Production	Export
India	90	22 (1994)
France	80	52
Spain	1,000	-
Japan	2,060	-
United States	200	-

It could be seen that from the vastness of the seaweed industry, especially agar the world over, it promises, a very bright future in India. Its rapid expansion that has been witnessed during sixties and seventies itself is an indication of growing awareness of agar's key role in many industries. The 1976 values of agar and carrageenan, and important allied products in present day seaweed industry, world wide are minimum of US $ 10 million and US $ 50 million respectively produced from 2 million and 20 million lbs of seaweeds (Gordon, 1976).

In India also the production of agar has made rapid strides although the resources are slender to meet the requirement of industry to work at full rated capacity. This is largely due to establishment of small and cottage scale production units, which in fact are more economical as far as agar is concerned. There is no increasing tendency amongst Indian seaweed industrialists to utilize alternative resources of agarophytes, which would definitely help its rapid expansion. A number of alternative resources of agarophytes have been repeatedly emphasized by Rama Rao (1974, 1977), Thomas (1977), Chennubhotla *et al.* (1977), Subba Rao *et al.* (1977) Levring *et al.* (1969), Chapman (1970), Krishnamurthy (1971) for more detailed information). The ways and means of conserving the present slender resouices of agarophytes, especially *Gelidiella*, the principal Indian agarophyte (Subbaramaiah, 1977) were also suggested. A good deal of research work was also carried out (Rama Rao & Subbaramaiah, 1979) on *Gelidiella acerosa* to evolve a suitable period of harvesting it, so that its natural growth is not impared. This technique, if adopted by commercial collectors as is done in America for *Gelidium*, will not only help to conserve its natural resources but can be easily applied to other agarophytes. Having realised the importance of augmenting the slender resources of agarophytes in the country, extensive work is going on to artificially cultivate them, which would certainly boost the agar industry in India CSMCRI marine algal Research Station, Mandapam Camp has developed viable technologies for artificial cultivation of *Gelidiella*, *Gracilaria* and *Hypnea* and is poised for take off on commercial scale. Besides, the extensive survey of agarophyte resources, presently being carried by Central Salt and Marine Chemicals

Research Institute, will go a long way in opening new vistas in Indian agar industry if these resources are exploited thoroughly but sensibly. In addition CSMCRI also is ready with technologies for production of agarose and carrageenan.

Acknowledgements

Author is grateful of Prof.P.Natarajan, Director Central Salt and Marine Chemicals Research Institute, Bhavnagar for his help and encouragement in bringing out this revised article. My special thanks are also due to the following persons: Dr.A.V.Rao, CSMCRI, (Late) Dr.F.J. Simpson, Director, National Research Council, Canada Atlantic Regional Laboratory, Halifix, Canada, Dr.J.V.Kingston, Division of Scientific Research and Higher Education, UNI, Paris, Dr.Kalus Hellenbran, Manager, Biology Division, Nova Scotia Research Foundation Corporation, Nova Scotia, Canada and Dr.Goran Michanek, Resident Representative, UNDP, Tripoli, Libya, who provided the author with some of the most valuable information on agar industry that helped to update the data, and also GATT, UNCTAD, ITC, Geneva, FAO Rome officials for providing with upto date data on trade of seaweeds and seaweed products. Last, but not the least is to express my thanks to Shri.P.K.Swamy, Asstt. Director, Marine Products Export Development Authority, Cochin for supplying me with the latest export figures for Indian agarophytes. I may fail in my duties take if I do not thank (Late) Shri K.S.H.M. Mohamed Meerasha Maraicayer, Pamban, Tamil Nadu, the pioneer seaweed supplier in India for giving valuable information on the present status of agar industry in India and also CPIL, Madurai.

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Some Endangered Medicinal Plants of Nepal

N.K.Bhattarai

National Herbarium, G.P.O. Box 938, Kathmandu, Nepal

Introduction

NEPAL extends along the great Himalayan range south-eastward from 80°15'E to 88°10'E longitudes, a distance of some 800 km, has an area of 141,000 sq.km where the altitude ranges from 60m to 8848m above the sea level. Being a mountainous country, it provides a great variety of habitats which is vividly reflected in the richness of its flora, estimated to comprise about 7000 species of higher plants (32). Species diversity in Nepal can be well illustrated from the fact that over 1000 species of Himalayan plants have been originally discovered and described from the Nepalese flora among which 252 species are endemic to the country (2).

Every year thousands of tonnes of medicinal herbs are collected from forest land and traded to foreign countries. The trade in medicinal herbs is an important source of revenue to the government and a major source of income to the rural people of the country. The medicinal plants in Nepal lists well over 700 species comprising about 10% of the known vascular plant species of the country (52). Among these, more than 100 species of medicinal herbs are currently exploited for the commercial purposes (53). Over 60% of the total export of herbs is to India with whom trade links have prevailed since time immemorial (51). The varied natural resource of medicinal herbs is not only an item of export, these also have been playing an important role in the health care of native people. About 85% of the rural Nepalese population still use traditional herbal remedies utilising the local plant

resource. These remedies are fairly well accepted, easily available, bearing a minimum cost and, in most of the cases, are the only available means (8). Therefore, native herbs play an invaluable role in the health services of the Nepalese population. However, medicinal herbs, Nepal's major export and foreign exchange earner, and the most vital source of folk medicaments, are in deep trouble, most of which are even endangered.

Causes of Threat

A. Deforestation

Tropical hardwood has dominated the trade based on natural resource in Nepal since more than a century and this trend has very recently declined because of the deplition of timber reserves from the natural forest through logging and conversion of forest land to other uses. Apart from industrial and commercial uses, forests are also destroyed as it is the major source of fuelwood and construction materials. Population pressure and the so-called developmental activities are causing a rapid deplition of forest in every part of the country. As the forests are destroyed, countless plant species face considerable danger of extinction.

B. Forest Fires

Occasionally, forests are subjected to fire to obtain dead trees for fuelwood and construction materials, and also to extend the area of the adjoining agricultural lands. Forest fires, especially those in the high-altitude pine (*Pinus wallichiana*) forests are set to enhance the growth and yield of the high priced morel mushrooms (*Morchella conica, Morchella esculenta*, etc.). The Pastures are regularly subjected to fire to produce tender grasses. As a consequence, many valuable herbs flourishing in those open habitats are indiscriminately destroyed. It also destroys the alpine butterfly belonging to the genus *Leptropteris* whose larvae are the host for the highly reputed medicinal fungus *Cordyceps sinensis*, ultimately threatening even the fungus. Mostly the fires are beyond control, resulting in severe loss of the forest, destroying the herbs, roots and rhizomes at ground level and even the clumps. Organic matter of the upper layer of the soil is usually destroyed resulting in the retardation or even the extinction of many valuable plant species in the area.

C. Shifting Cultivation

The shifting cultivation, another form of destruction of forests, called 'Khorea phandne' is identical to the 'Jhumming' system of agriculture practiced in Meghalaya, India (69). A patch of the climax forest is destroyed, burnt and used for farming for some years after which it is abondoned and the farmers shift to another forest area, cleaning another patch of the climax vegetation. This practice results in the destroying of a large number of biological species and the abondoned land is mostly succeeded by a few number of sporadic species that are quite different from the original vegetal cover.

D. Over-grazing

The livestock population in rural localities exceeds far more the human population and the shortage of fodder and other feeding materials resulting in the over-grazing in the pastures has been contributing not only to the deplition of natural vegetal cover but also to the retardation of plants including the medicinal herbs in the region.

E. Over-exploitation

Medicinal herbs are regarded as a free commodity to be collected from nature. Every year thousands of tonnes of medicinal herbs including roots and rhizomes are collected from forest land and traded to foreign countries. The trade in non-timber forest products has long been an important source of income to the rural people of the country, estimated in the order of ten million US dollars per year (28).

The traders provide easy credit to the rural people and are interested in obtaining the largest volume possible. The local collectors therefore find themselves in debt to the traders and tò pay the debt must meet the collection demands of the traders. The consequence, raw materials are over-harvested when, for example, immature plants, roots, tubers and rhizomes are taken or overpruning are done. This destroys the region's natural resources and gradually particular species have become more difficult to find in a given locality where once they flourished.

Apart from the commercial purposes, a large number of plants that are regularly harvested for local uses like medicine, food, fodder, fibre, dye, tan, gum, etc., also have severely affected the natural regeneration, habitat destruction and the very existance of many medicinal herbs in many areas.

Some Endangered Medicinal Plants of Nepal

In the following enumeration fourteen medicinal plants, supposed to be most endangered in Nepal due to various factors mentioned earlier, has been dealt in an alphabetical order according to their Latin names. For each species, its common synoname and family are given in succession. It is followed by the common names of the species consisting of English (E), Hindi (H), Nepali (N), and Sanskrit (S) names, where available. A brief account of the habit and habitat of each plant is given, followed by the phytochemical characteristics and a review on medicinal uses with literature sources. The voucher specimens of the considered plants, collected by the author in different parts of the country, can be found in the National Herbarium (KATH), located at Godawari, Lalitpur district, Nepal.

1. *Acorus calamus* L.; Araceae

Common names: Sweet flag (E), Bach, Ghorabach (H), Bojho (N). Vacha (S).

A semi-aquatic perennial found in the tropical to the temperate regions (200m-3000m) in marshy and moist situations.

Chemical Constituents

The rhizome contains glucosides scarin and acorin, and essential oil; the essential oil contains calamen, calamenol, calamenon ascarone, asaryl aldehyde, eugenol, pinene and camphene (24,30,74).

Medicinal Uses

A piece of the rootstock is chewed to relieve throat infection (5,7,9,10,13,16,37); it is said to act as an expectorant (13); it is also used to relieve cough and colds (1,18,26,45,46,56,61); it is also used as a mesticatory for toothache (66).

A paste of the rootstock is considered antiseptic to wounds in man (9) and cattle (6,45,46).

A warm decoction of the rootstock is used to wash the mouth to treal gingivitis, pyorrhoea and scury (9).

A warm decoction of the rootstock is sipped to exhibit anthelmintic action (5,7,10,12,37); it is also taken to relieve bronchitis (16, 45, 46). The rhizome is taken to cure fever dysentry, brenditis & asthma (10, 26, 79); it is also considered useful in abdominal pains (18, 26, 48) inflammations, neck pains & to improve appetite (38, 48).

The rhizome juice mixed with sesame oil (*Sesamum indicum* L.) is massaged over chest to relieve chest pains (38).

The rhizome is carminative, emetic, expectorant, tonic and stimulant. It is said to be effective in dysentery and snake bite (74,92).

The rootstock is crushed with salt, diluted in water and porescribed to cattle to relieve dyspepsia (11).

The rootstock is boiled with goat's milk and drunk to relieve infantile diarrhoea (14).

The rootstock is crushed with honey and orally prescribed to children to improve vocabulary (14).

The rootstock is crushed with rape oil (*Brassica rapa* L.) and applied on the anus to relieve piles (16).

The rootstock is crushed with neem leaves (*Azadirachta indica* A. Juss.) and eaten to facilitate expectoration and to relieve sore throats (13).

A decoction of the rootstock prepared together with neem leaves is said to act as an expectorant. It is said to possess antipyretic property and also used to relieve colic pains (16).

It is an aromatic bitter tonic and carminative; it possesses emetic and anti-spasmodic properties (1,18,26); it produces beneficial results in cases of dyspepsia and chronic diarrhoea (1).

Powdered rhizome is used as vermifuge (68,81).

Alcoholic extract of the plant has sedative and analgesic effects and causes moderate depression in blood pressure (81).

Rhizome form an ingredient of several Ayurvedic and Unani medicinal preparations (42).

Other Uses

The powdered rhizome possesses insecticidal properties and useful against bed-bugs, moths, lice, etc (1,48,68,74,81).

The crushed plant is used to wash head to kill lice (61,86); rhizomes are used in flavouring gin, beer, drinks and other foodstuffs (81,89); essential oil obtained from the leaves is used by perfumers in the manufacture of hair powder (85,89).

2. *Asparagus racemosus* Willd.; Liliaceae

Common names; Asparagus (E), Satavari, Satawar, Satmuli (H), Kurilo, Satawari (N), Shatavari (S).

A woody much branched, spinous climber found in the tropical to temperate regions (200m - 3000m) in forests and shrubberies.

Chemical Constituents

Plant contains sarsapogenin and glucoside (23,41).

Medicinal Uses

The tender shoots are eaten raw as an antipyretic drug (6,10).

A decoction of the tender shoots is considered a vital tonic which is prescribed to convolescents and post-natal mothers (9,10,17,57).

Decoction of the bark is used in amoebic dysentery (63).

The root paste is eaten as an antipyretic drug (6,10,34,50); it is also used as an antiseptic to wounds (20).

The root powder is given to women during labour pains to facilitiate delivery (55).

The root decoction is taken to relieve diarrhoea (14,91) and dysuria (16); it is also considered a vital tonic (14,77); it is also eaten to relieve stomach complaints (35,54,83).

The semi-roasted root is taken with milk to relieve dysuria (16)

The root is used medicinally as a refrigerant, demulcent, diuretic, aphrodisiac, antispasmodic, alterative, antidiarrhoeatic and antidysenteric (18,20,22,89,92).

The root is used by native physicians as a stimulant and restorative (89).

The root is said to be tonic and diuretic and useful as a galactagogue (1,26,46,47,68,73,80,92).

Roots are used as an ingredient in the preparation for treating impotency (18,34,67); they are used as an aphrodisiac and tonic (4,18,26); it is taken with milk to initiate conception (18,82), and also considered an abortifacient (82).

Roots are used as an aphrodisiac and tonic (4,18,26); they are also used in piles and diseases of eyes (91).

Root extract is given as a nerve sedative (21); it is also dropped in the mouth or nose to stop bleeding (44,84).

Root is useful in leprosy, rheumatism, gonorrhoea and tuberculosis (26), and also used as a remedy against amenorrhoea (18).

A mixture of honey and fresh root is given as a demulcent in dyspepsia (1,68,92).

Root pounded with other plant products is drunk to cure bloody urination (3,20,36) and other urinary complaints (18).

The root is largely used in the preparation of medicated oils, prescribed for nervous and rheumatic complaints (1,91). It is also used as an ingredient of many medicines (18).

The root is used as a demulcent in veterinary medicine (22,81,89).

The fruits are eaten to treat pimples (59).

Other Uses

Tender shoots are cooked like vegetables (9,10); roots are boiled and eaten (46); fresh root is used for the preparation of "Ranu", material used for fermentation (20).

3. *Cordyceps sinensis* (Berk.) Sacc.; Hypocreaceae

Common names: Yarsagumba, Jiwanbuti) (N).

A fungus parasitic in a caterpillar (*Lepidopteris* sp.) found in the alpine open slopes (4300m-5000m).

Medicinal Uses

The fruiting bodies are eaten as an aphrodisiac and a tonic (6,9,15,17,71); mostly it is eaten with honey or milk (9).

The fruiting bodies are prescribed to cattle, especially to yaks, as an aphrodisiac (11).

4. *Dactylorhiza hatagirea* (D.Don) Soo; syn. *Orchis latifolia* L.; Orchidaceae

Common names: Orchis (E), Salap (H), Panchaunle, Hastajadi (N).

A perennial ground orchid found in the temperate and sub-alpine Himalaya (2800m-4000m) in shrubberies, open slopes and marshes.

Chemical Constituents

Rhizome contains glucoside (62).

Medicinal Uses

A paste from the rhizome is applied to cuts and wounds for the rapid healing action (6,9,15).

The rhizome are boiled and eaten as a remedy for various types of abdominal pains (9,15).

The rhizome is eaten with honey or milk as an aphrodisiac and a tonic (6,9,15,17,62,74,86,88).

The tuber is expectorant and astringent (1,62,74,88).

The tuber is supposed to be nutritious and helpful in diarrhoea, dysentery and chronic fevers (1,88).

The tuberous roots, finely powdered and boiled with milk, form a nutritious article of diet and is given in phthisis, diabetes, chronic diarrhoea and dysentery (22).

Salep of commerce, consisting of washed, scalded and dried tubers, are used as farinaceous food, nervine tonic and aphrodisiac (1).

Other Uses

Tubers are eaten raw or boiled (6,9).

5. *Daiswa polyphylla* (Sm.) Rafn. var. *wallichii* Hara; Syn. *Paris polyphylla* J.E. Smith; Liliaceae

Common name: Satuwa (N)

Chemical Constituents

Rhizome contains two glucosides, a-paridin and a-paristyphnin, and sugars (1)

Medicinal Uses

A piece of the root is chewed to heal "internal" wounds below the throat (8).

The root paste is applied for rapid healing of wounds (9,19), boils and carbuncles (15). It is also applied as an antidote to the bites of poisonous snakes and insects; it is also eaten to alleviate narcotic effects (12).

The root paste is applied to treat boils and carbuncles (15); it is also applied to heal open wounds (9).

The root paste is eaten with honey and milk as a galactagogue and a tonic (1,8).

The root powder is taken as an anthelmintic drug (1,56); it is also considered useful in fever and diarrhoea (86).

A decoction of the root is drunk as an anthelmintic and a febrifuge (9).

The glucoside a-paristyphin has a depressant action on carotid, myocardium and respiratory movements (81).

6. *Fritillaria cirrhosa* D. Don; Liliaceae

Common name: Kakuli(N)

A herbaceous plant found in the temperate to alpine Himalaya (2700m-4000m) in open slopes and shrubberies.

Medicinal Uses

The bulbs are made into a paste and applied to wounds as an antiseptic drug (15,71); it is also eaten to relieve abdominal pains (15).

The dried corms are given in asthma, bronchitis, and tuberculosis (1,22,42,81).

7. *Nardostachys grandiflora* DC.; Syn. *N. jatamansi* DC.; Valerianaceae

Common names: Spikenard (E), Jatamansi, Balchhar (H), Jatamansi, Bhulte (N), Jatamansi (S).

An aromatic perennial herb found in the sub-alpine and alpine regions (3600m-4500m) on screes and rocky slopes.

Chemical Constituents

The rhizome and roots contain volatile essential oil, resin, sugar, starch, bitter extractive matter and gum (1).

Medicinal Uses

The rhizome paste is eaten as a bitter tonic (9,48,62); it is also eaten to treat dysentery (9).

The rhizome paste is applied to treat piles (55,56).

The rhizome is considered tonic, stimulant, antispasmodic, diuretic, deobstruent, emmenagogue, stomachic and laxative (1,18,26,62).

An infusion of the rhizome is reported to be useful in epilepsy, hysteria, palpitation of heart and cholera (1,62).

A tincture of the rhizome is given in intestinal colic and faltulence (1,62).

The rhizome is said to have antiseptic (22) and haemostatic (48) properties.

Rhizomes are used as a diuretic, blood purifier, in coughs, ulcers and snake bites (48); it is considered to be beneficial in low blood pressure (18). The rhizome is considered to have anthelmintic properties, and also considered useful in cholera and jaundice (26).

A tincture of the rhizome is used for treating epilepsy, hysteria and convulsions; it is also used as laxative and for improving urination, regulating menstruation and digestion(18,26,81).

The rhizome is used as an aromatic adjunct in the preparation of medicinal oils (1,18,89); it is reported to promote the growth of hair and also impart blackness (1,18,26,81,86).

The essential oil possesses antiarrhythmic activity with possible therapeutic usefulness in cases of auricular flutter (1).

The plant is said to possess all the properties of official valerian *(Valeriana officinalis* L.) and used as a substitute for the same (1,62).

Other Uses

The essential oil is largely used in perfumery (48,81); it is an ingredient of hair washes and hair oils (26).

The rootstock is used as an auxillary in dyeing along with *Mallotus philippensis* Muell.-Arg.(89).

8. *Picrorhiza scrophulariiflora* Pennell; Syn. *P. kurroa* Royle ex Benth.; Scrophulariaceae

Common names: Hellebore (E), Katki, Kuru (H), Kutki, Katuki (N), Katula, Katuka, Katurohini (S).

A perennial herb found in the sub-alpine and alpine regions (3600m-4500m) on screes and rocky slopes.

Chemical Constituents

Roots contain picrorhizin, kutkin, kurrin, vanillic acid, kutkiol, kutkisterol and picroliv (1,78,87). The constitution of kutkin, the bitter glucoside, has been studied in detail (70).

Medicinal Uses

The root paste is applied to heal wounds in cattle (6,11).

Roots are used in abdominal pains (48,62,74,86,88), and as a laxative (69) and purgative (62,74,86). They are said to exhibit anthelmintic properties (18), and are also used in dyspepsia (62).

Roots are useful in asthma (18,48,88) and jaundice (48,88); they are also used in scorpion sting (62).

The root decoction is drunk as an appetizer (6,9,15,17,48), especially to overcome anorexia (9,17); it is considered to be a tonic (6,15,18,55,71); it is drunk as an antipyretic drug (6,9,5,18,48,55,62,65,74,76); it is taken to relieve cough and colds, supposed to act as an expectorant (13,15,18).

The root decoction is considered to have galactagogue (12) and anthelmintic (12,76) properties.

Roots are used as stomachic, tonic and improves appetite and stimulates gastric secretions (65,81).

It is considered to be a valuable bitter tonic, almost as effecacious as gentian, *Gentiana kurroo* Royle. Dried rhizomes and roots is used either as an adulterant of or as a substitute for Indian gentian, *Gentiana kurroo* (1).

It is antiperiodic, cholagogue, stomachic, laxative in small doses and cathartic in large doses; it is reported to have beneficial action in dropsy (1,62).

The root decoction is given with salt to febrile cattle as a febrifuge (II); roots are used traditionally in animal treatment (33).

9. *Podophyllum hexandrum* Royle; Syn. *P. emodi* Wall.; Berberidaceae

Common names: Himalayan May apple (E), Laghupatra (N.S), Papra (H).

A perennial herb found in the temperate to alpine Himalaya (2400m-4500m) in forests, shrubberies and open slopes.

Chemical Constituents

The rhizome contains podophyllin, quercetin, kaempferol, astragalin, an essential oil, wax, and mineral salts. Podophyllin contains podophyllotoxin as the major active constituent (1). Chemical investigation revealed that the constituents of the rhizomes of Indian podophyllum, *Podophyllum hexandrum*, are identical with those of *Podophyllum peltatum*, the American podophyllum (27).

Medicinal Uses

The ripe fruits considered to be a mild laxative (9).

Rhizomes and roots are used in fever (18,65); they are also considered stimulant and used for increasing bils secretion (92).

The rhizome paste is applied to treat gynaecological complaints (9); it is also eaten as a purgative (9,18).

The dried rhizomes and roots of *Podophyllum hexandrum* are official in I.P. and B.P. (1,19,22) and form the source of a medicinal resin, podophyllin. Podophyllin is listed in various other pharmacopoeias and formulations (1).

During recent years, the resin podophyllin has acquired special importance for its possible use in controlling some forms of cancer (1,62,81,88,92).

Podophyllin is used as stimulant and purgative (1,39,74,81); it is considered a cholagogue, alterative, emertic and bitter tonic (1).

Podophyllin is also used in veterinary medicine as a cathartic for dogs and cats; an ointment of podophyllin is employed to remove warts in animals (1).

Other Uses

The ripe fruits are edible (86,89); seeds are used in fermenting a country liquor (76).

10. *Rauvolfia serpentina* (L.) Benth. ex Kurz.; Apocynaceae

Common names: Serpentine (E), Chandrabhaga, Sarap gandha, Chota- chand (H), Sarpagandha, Chandmaruwa (N), Sarpagandha, Chandrika (S).

An erect evergreen, small shrub found in the tropical region (upto 1000m) in forests.

Chemical Constituents

Root contains a large number of alkaloids, oleoresin, starch and calcium oxalate (90).

Medicinal Uses

The root paste is applied and eaten as an antidote to the bites of poisonous reptiles and stings of insects (8,18,45,46).

The root paste is eaten to cure malarial fever (8,43,58); it is also considered to be a general febrifuge (8,18,26,45,46,48). It is considered a bitter tonic and narcotic (18).

Extracts of the root are valued for the treatment of intestinal disorders, particularly diarrhoea, dysentery (1,90,26) and also as anthelmintic (1).

Root extract is used in treatment of mental diseases (21,72,92); it is also given in guiddiness (21). It is given in high blood pressure (18).

Decoction of roots and leaves is given to stop vomitting (43).

Mixed with other plants the roots have been used in the treatment of cholera, colic and fever (1,81).

Juice of leaves is used as remedy for opacity of cornea (18,65,81).

In recent years, *Rauvolfia serpentina* and its preparations have become important therapeutic agent, both as antihypertensive and as a sedative (1,21,65,81,92); it is employed for relief of various central nervous system disorders associated with psychosis, schizophrenia, insanity, insomnia and epilepsy (18,26); extracts of roots are valued for treatment of intestinal disorders; roots are reported to stimulate uterine contraction and is used in child birth (18,25,65,81,92).

Rauvolfia serpentina is an official drug in I.P. (22).

11. *Rheum australe* D.Don; Syn. *R. emodi* Wall. ex Meisn.; Polygonaceae

Common names: Indian rhubarb, Himalayan rhubarb (E), Ravand chini (H), Padamchal, Padamchalnu (N), Amlavetasa (S).

Tall perennial found in the sub-alpine and alpine Himalaya (3000m-4200m) on open slopes.·

Chemical Constituents

Root contains emodin, a glucoside rhapontien, tannin, resins, mucilage, sugar, starch, pectin, lignin, calcium oxalate and organic salts (1,24). Leaves and stem contain oxalic acid, leaves and flowers contain rutin (1).

Medicinal Uses

The petioles are eaten with salt as an appetizer (6,9).

The rhizome paste is used to massage the affected parts to treat rheumatism (9,37).

The rhizome paste is used to plaster the affected parts to treat sprains and swellings in man (76,86) and cattle (II).

The rhizome paste is eaten with milk as a purgative (9,56).

It is used as a purgative, astringent, tonic and also in dyspepsia (1,18,81); it is also used in diarrhoea (18).

Root powder is sprinkled over ulcers for quick healing (1,76,86); rhizome paste is applied on cuts for the accelerated healing action (76,86); it is also used in many veterinary complaints (33).

Rheum australe is official in I.P. (22).

Other Uses

Powdered roots are used for cleaning teeth (1).

Roots with madder and potash are used for dyeing fabrics red (1,81,89).

The petioles, leaves, leaf - stocks and flowers are edible (81,89).

12. *Rubia manjith* Roxb. ex Fleming; Syn. *R. cordifolia* L.; Rubiaceae

Common names: Indian madder (E), Manjit, Manjith (H), Majitho (N), Manjistha, Kala-meshika (S).

A herbaceous climbing perennial found in the sub-tropical to temperate Himalaya (1200m-2700m) in forests and marginal lands.

Chemical Constituents

Roots contain resinous and extractive matter, gum, sugar, salts of lime, and colouring matter. Colouring matter consists of purpurin, majistin, garanein, alizarin and xanthine (1,24,29,39). The root also contain small amounts of purpuroxanthin and pseudopurpurin (1).

Medicinal Uses

The plant is used in rheumatism, pleurisy and other chest complaints (31,92).

The root is used as an astringent and tonic (18,49,60); it is also given in urinary troubles (64).

The roots are useful in eye sores, liver complaints and spleen enlargement (31); they are also used internally to treat menorrhagia and other menstrual complaints (31,92).

Root decoction is used in paralysis and jaundice (92).

Roots and fruits are used in paralysis, ulcers and skin diseases (49); the stem is used in cobra bite and scorpion sting (49,60); crushed roots are applied for poisonous stings of insects and caterpillars (69,92).

The root juice, mixed with ginger, is boiled in milk and drunk warm to relieve menostaxix (16).

A decoction of the leaves and stem is used as a vermifuge (1,22,81); crushed leaves are applied for ulcers (69).

Roots form an ingredient of several Ayurvedic preparations (1,81).

Other Uses

It is employed for dyeing cotton fabrics, blankets and carpets (1,18,64,81). The root is also used for colouring medicinal oils (1).

13. *Swertia chirayita* (Roxb. ex Fleming) Karsten; Syn. *S. chirata (Wall.) C.B. Clarke; Gentianaceae*

Common names: Chirata, Chiretta (E), Chireta, Kiryat-charayatah (H), Chitaito (N), Kirata-tikta, Bhunimba (S).

An erect annual herb found in the sub-tropical to temperate Himalaya (1200m-3000m) in shrubberies and open lands.

Chemical Constituents

Plant contains two bitter principles, ophelic acid and chiratogenin, two bitter glucosides, chiratin and amarogentin, gentiopicrin, two yellow crystalline phenols, swerchirin, resin, tannin, and ash (1,24,75).

Medicinal Uses

A decoction of the aerial parts is taken as an antipyretic drug (6,48,56,76); it is also taken to relieve various types of body aches (6).

It is considered a tonic (18,39,48,74,76), febrifuge (18,26, 47,48, stomachic, laxative (18,26,42,74,81), anthelmintic, antidiarrhoeal (42), useful in anaemia, bronchial asthma and liver disorders (81).

The plant is used in intermittent fevers and bilious dyspepsia accompanied by fever (18); it is also used in skin diseases (18,26,48,76).

The plant is considered stomachic, anthelmintic, appetizer, alterative, antidiarrhoeic, and also considered beneficial in bronchial asthma (26).

The dried twigs are used as a remedy in malarial fever (31,74); it is also used as a blood purifier (48,76).

It is an effective substitute for gentian (*Gentiana kurroo*)

It is official in I.P. and was formerly also official in B.P. and U.S.P. (1,22,89).

Other Uses

Chiretta is used for dyeing cotton cloth yellow. It is also used in the liquor industry as a bitter ingredient (1).

14. *Valeriana jatamansi* Jones; Syn. *V. wallichii* DC.; Valerianaceae

Common names: Indian valerian (E), Tagar, Mushkbala (H), Sugandhawala, Samayo (N), Tagara (S).

Perennial herb found in the sub-tropical to sub-alpine Himalaya (1500m-3600m) in forests, shrubberies and open slopes.

Chemical Constituents

The rhizome contains tartaric, citric, malic, and succinic acids and a volatile oil (23).

Medicinal Uses

The root paste is applied on the forehead to relieve severe headache (9,56); it is also applied to treat various eye problems (56).

Roots are stimulant, carminative, antiseptic, effective in epilepsy, neurosis and hysteria (40,74), they are also used in diseases of eye, blood, liver, spleen and nervous system; bath of valerian is useful in rheumatism (31).

The essential oil has tranquilizing properties (39).

The root juice is used as a nasal drop to overcome nose-bleed (9); it is also applied to treat gout (55).

The root juice is diluted in water and used to bath children to relieve "weeping sickness" (8).

It is prescribed as a remedy for hysteria, hypochondriasis, nervous unrest and emotional troubles (1,81).

The rhizome is used as a carminative (1,40,74); powdered rhizome, mixed with sugar, is used in urinary troubles; a decoction of the drug is given to mothers after parturition, probably as a sedative (1).

It forms an ingredient of a number of Ayurvedic recipes (1,81,89).

Valeriana jatamansi is official in I.P. (22).

The plant is said to possess all the properties of official valerian (*Valeriana officinalis* L.), and used as a substitute for the same (41).

Other Uses

The rhizome is employed for hair oils and perfumes (1,81,86), as incense (1,81) and as an insect repellant (1).

Conclusion

A large number of the Nepalese medicinal plants bear varied degrees of domestic, therapeutic and industrial potentialities which have been playing an important role in the health services and livelihood of the rural population. The interaction between the activities of these people and the plant resources has emerged as a critical factor in sustaining the region.

Nepal is principally an agricultural country. With few off-farm employment opportunities, the increasing pressure upon the plant resources has grown beyond the carrying capacity of many areas. Destruction of habitat fueled by indiscriminate collection, not in accordance with any regulatory procedure or recognized management practices, has threatened the survival of some species and reduced the quality of many more. Poverty and its bedfellow illiteracy have forced the rural Nepalese people to continue activities which help them survive in the present but which will cause more severe problems in the future. When people must devote most of their energies today to assure food for tomorrow, it is difficult to conserve and direct resources for the benefit of the next generation. The destruction of habitat and excessive collection of medicinal plant resources by the rural Nepalese people, therefore, is not only a case of preference but also of a situation of having no choice.

If the Nepalese medicinal plants are to continue to serve the needs of the people without being reduced to a dangerously unstable resourcebase, it has to be considered in the perspective of sound ecological management, and habitat conservation, leading to biodiversity conservation, should go together with the task of meeting the basic needs of the majority of rural Nepalese population. Consequently, sustainable development and conservation must be firmly linked if Nepal is to meet the needs and improve the quality of life of its present population and future generations.

Lastly, the present paper and the enumerated medicinal plants should not be considered as the final word on the topic, but rather as a point as a point of departure for further discussion and intellectual formulations.

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Traditional Medicine

P. Pushpangadan

Tropical Botanic Garden & Research Institute, Pacha-Palode
Thiruvananthapuram - 695 562, Kerala

SEARCH for eternal health and longevity and to seek remedy to relieve pain and discomfort prompted man to develop diverse ways and means of health care. The early man explored his immediate natural surroundings and tried many things like plants, animals and minerals and developed a variety of therapeutic agents and tonics. Over millennia that followed the most effective agents amongst them were selected by the process of trial or error, empirical reasoning and even after experimentation. This effort has gone in history by the name medicine. The knowledge gathered by generations was passed on to the posterity and this practice is generally termed as 'Traditional medicine' or 'Ethnomedicine'. World Health Organization (WHO) refers to traditional medicine as a vague term loosely used to distinguish ancient and culture bound health care practices which existed before the application of science to health care practices.

The primitive man combined instinct with his indulgence and learned many lessons from other animals in the art of healing. All living creatures including the plants, find ways and means to ward off decay and diseases, to conquer discomfort and disease and postpone a day of their demise. In fact the conscious selection of plants for use as drugs probably began with the dawn of settled civilizations. The empirical search for agents to cure diseases probably began with the earliest stirring of humanoid creatures, the fore runner of the Homo sapiens.

In many eastern cultures such as those of India, China and Arab world this experience was systematically recorded and incorporated into regular system of medicine that developed and became part of the Materia Medica of the Traditional System of Medicine of these countries. The ancient civilizations of India, China,

Greece, Arab and others developed their systems of medicine independent of each other and all of them were predominantly plant based. In fact, unconscious selection of plants for use as drugs probably began even before the dawn of settled civilizations. The traditional systems of medicine are very much alive even today over large parts of the globe.

In 1976 the World Health Assembly took note of the vital role that Traditional Medicine play in health service, particularly the remote areas and draw attention to the manpower reserve constituted by the traditional practitioners (resolution WHA 29.72). In 1977 the WHO adopted a resolution to declare that the main social target of Governments in the coming decades should be the attainment by all people of the world, a level of health permitting them to lead socially and economically productive life known popularly as the goal of 'health for all' by 2000 AD. Resolution WHA 30.49 urged countries to utilize their traditional system of medicine. The Alma Ata conference of WHO in 1978 further declared that the primary health care is the key in attaining this goal and identified the vital role of the traditional medicine in achieving the same.

It has been estimated that 80% of the people living in developing countries are almost completely dependent on traditional medical practices for their primary health care needs, and higher plants are known to be the main source of drug therapy in traditional medicine. Since about 80% of the total world's population reside in developing countries, about 64% of the total population of the world utilize plants as drugs i.e. 3.2 billion people (Farnsworth, 1990).

Traditional medicine is essentially the sum total of all knowledge and practices - that can be explained or noted - used in the prevention diagnosis and elimination of physical, mental and social impairment. It relies exclusively on practical experience of observation and is handed over from generation to generation. Traditional medicine includes a wide spectrum of such medical knowledge system like folk remedies, tribal medicine, ayurveda, sidha, unani, amchi, acupuncture, massage therapy, herbalism, yoga therapy, spiritual therapy, bone setting etc. Traditional medicine is a heterogeneous term that refers to a broad range of ancient and natural health care practices which existed before the application of the modern scientific method to health care. Until the beginning of the 19th century all medical practices were what we now call the traditional medicine. With the renaissance there came the great scientific upheaval in most spheres of human activities and began the introduction of cartesian scientific materialism into all human activities, notably in the theory and practice of health care. Its metod was to break up complex phenomenon into their component parts and deal with each one in isolation (Pushpangadan, 1995). By contrast, traditional medicine attempts to embody a holistic approach i.e. viewing an individual in his totallty within the socio-ecological system. The fact is that whatever may be the approach, the goal of medical system, traditional or modern remains one and the same i.e., improving the health and quality of life of mankind.

It is now well understood that any health care system to be meaningful and effective it must be culturally, socially and environmentally closer to the masses. This is particularly true for meeting the primary health care needs of the rural and tribal people. A significant aspect of the local health traditions and their practitioners is their self-reliant nature. These traditions are entirely autonomous in character and rooted deep in the community's social tradition and knowledge system. Perhaps this is one of the reasons why WHO, while declaring the Health for All by 2000 AD, emphasised the strategic role that the traditional medicine has to play in achieving this goal, especially in meeting the health care needs of the rural and tribal populations of the world.

Traditional Medicine in India

The Indian subcontinent is endowed with one of the richest expertises in traditional medicine. The traditional medicine in India functions through two social streams. One is the local folk stream (the local health traditions) which is prevalent at villages and tribal set-ups in India. The carriers of these traditions are millions of house wives, thousands of traditional birth attendants, bone setters, practitioners in accupressure, eye treatment, snake bites treatment etc. and the traditional village level herbal physician, the 'vaidyas' or tribal physicians in the tribal areas. Table 1 gives the details of the folk medicine stream in India. These local health traditions thus represent an autonomous community system of health delivery at the village level which runs parallel to the state supported system. Its potential goes largely unnoticed because of the dominant western medicine.

A second level of traditional health system is the scientific or classic system. This consists of codified and organized medical wisdom with sophisticated theoretical foundations and philosophical explanations and expressed in thousands of regional manuscripts covering treatises on all branches of medicine and surgery. Systems like Ayurveda, Sidha, Unani, Amchi and Tibetan are the expressions of this stream.

Ayurveda is perhaps the oldest (6000 BC) among the organised traditional medicine. It has gone through several stages of development in its long history. It spread with Vedic and Hindu culture as far east as Indonesia and to the west it influenced the ancient Greek, who developed a similar form of medicine. The Buddhists added many new insights to it and they took it along with their religion to many different countries. In this way, Ayurveda became the basis of the healing tradition of Tibet, Sri Lanka, Burma and other Buddhist lands and influenced Chinese and Greek medicine. Ayurveda is thus a rich tradition, adaptable to many different times, cultures and climates.

Ayurvedic healing has two levels; one for the layman and self care, the other for the health care professional, the physician. The first outlines a general constitution or life style treatment for health enhancement and disease prevention. It has many common home remedies for different diseases. It is important to realize that

Table 1– *Folk-medicine stream carriers of village based health traditions

Traditional Carrier	Subjects	Nos.
Housewives and elders	Home remedies Food and nutrition	Millions
Traditional birth attendants	Normal deliveries	7 lakhs
Herbal healers	Common ailments	3 lakhs
Bone-setters	Orthopaedics	60,000
Visha Vaidhyas (Snake, Scorpion, Dog)	Natural poisons	60,000
Specialists	*Nethra *Skin *Respiratory *Dental *Arthritis *Mental Diseases *Liver *GIT *Wounds Fistula Piles	1000 in each area

* *Source* - Foundation for Revitalization of Local Health Traditions

many of our diseases can best be treated by ourselves. Often a few simple therapies done as part of our daily regime can be effective. It is only when our life style is out of harmony that more severe diseases arise, and more specialized and complicated health care becomes necessary.

The second level provides some of this specialized Ayurvedic medical knowledge and outlines more technical and more sophisticated and complicated remedies handed by a health care professional.

According to Ayurveda there are three primary life forces or three biological humors in the body - Vata, Pitta and Kapha. They correspond primarily to the element of air, fire and water. Ayurvedic diagnosis and treatment of diseases are based on these three biological humors. The proportion of the humors varies according to the individual. One humor will usually predominate and its nature will make its mark upon the organism in terms of its appearance and disposition. Most diseases arise from the excesses brought about by the inborn predominant humor and of Ayurvedic medicine prescribe methods for balancing or correcting the constitution. This constitutional approach is the essence of Ayurveda. It gives Ayurveda immense power for disease prevention, health maintenance, longevity enhancement, and the treatment of disease.

Ayurvedic masters explained that the process of life is in constant state of flux and not in a static condition and that in this dynamic process a continual adjustment with the environment is necessary for the health and well being. Sushruta and Charaka, the authors of the classical Ayurvedic texts 'Sushruta Samhita' (BC 2500) and ‹Charaka Samhita' (BC 1500) respectively, commented that if an organism fails to adjust and adapt either due to some innate deficiencies or due to overwhelming force of the environment the result is the diseased condition.

Charaka seems to have a combined role of philosophor and physician where as Sushruta who tried to free medicine from priestly dimension and created an atmosphere of independent thinking and investigation which later characterized the Greek Medicine. Sushruta can be acknowledged as one of the greatest medical scientists rather surgeons or physicians in the ancient India, who, for the first time changed the art of surgery into a practical science in the remote antiquity. Sushruta thus deserves the legitimate right to the little Father of Surgery.

During the second century BC Vagbhatta a Buddhist from Sind, wrote 'Ashatanga Samhita', that combined the essence of both the text, of Charaka and Sushruta. One of his descendants Vagbhatta Junior (8th century) presented all the knowledge of Ayurveda as it existed during his time in lucid manner in his 'Ashtangahritaya Samhita'. He dealt with surgery in detail as presented in the Sushruta Samhita. During the early medieval period Ayurvedic literature remained stagnant except for the work of Madhavacharya of 8th century.

The successive onslaught of the early foreign invaders caused total devastation of the medicinal education institutions in India. Ayurveda suffered greatly on this account. The continued growth and development of the Ayurvedic medicine was thus adversely affected. It is still not fully recovered from this stagnation.

Sidha medicine practiced mainly in Tamil Nadu and part of Kerala is considered to be the oldest system of medicine in India. This system traces its origin to Sidhars (saintly figures who get revelations/enlightments in medicine through yoga practice and Sidha spiritual achievement. All Sidha literature are in Tamil. It has also been referred to as the Agasthyar system, whereby its exponent sage Agasthya is tributed. The system developed in the prevedic Dravidian culture (4000BC) and after the introduction of Ayurveda the two systems seem to have developed parallel with similar principles and doctorines. Siddha specializes, to a larger extent on iatrochemistry and the usage of metals and minerals is much advocated in addition to that of medicinal plants.

The Unani system originated in Greece around 400BC and its name can be traced from the Arabic name for Greece, Unan. A number of Greek (e.g. Hippocrates, Aristotle, Herophilus) and Arabic (e.g. Ib-Bin Sina, Al-Mamum, Ibn Masawayh, Rhazas) philolospers have contributed to this system. Ayurvedic influence in the Greek as well as the Unani system can be noticed particularly in the

humoral concept of health and diseases. Unani was introduced in India about 1000 years ago with the coming of Mughal invaders.

The Amchi system or the Tibetan system of medicine prevalent in different Himalayan regions starting from Lahul Spiti and Leh - Ladhak to Shillong and Sikkim can trace its origin to the Ayurvedic system. Its therapy, however, makes use of more of animal and mineral products in addition to the herbs. Use of spring and mineral waters, moxibustion, mysticism and spiritual healings are also common in Amchi system.

While the classical traditions are documented in the form of texts or plam leaf manuscripts, most of the local health traditions, particularly the tribal medicine are purely oral in tradition. Of late, the traditional tribal communities in the world over are fast being engulfed in the rising tide of modernisation and they are giving up their age old medicinal practices. It was in this context the Ministry of Environment & Forests, Govt. of India launched an All India Coordinated Research Project on Ethnobiology (AICRPE). This multidisciplinary and multi-institutional project programme during the past 12 years has generated much valuable information on the rich and varied tribal medicine of our country. It has recorded over 7500 wild plant species used for medicine by tribals of India. The medicinal value of over 4000 plant species out of these some are either little known or hitherto unknown. Many of these plants are rare, endemic and are threatened of extinction on account of wide scale destruction of the forests.

Decline of Traditional Medicine

The introduction of abstract medicine in the form of base chemicals and pharmaceuticals during the 18th and 19th centuries has demonstrated method for bringing quick relief from sufferings and this won instant admiration and popularity. This system known as allopathy made rapid advances during the 19th and 20th centuries as a result of the advances made in biological, chemical and pharmacological sciences. New discoveries of sulpha drugs, synthetics, antibiotics, cortisones and other chemotherapeutic agents in quick succession swept all other systems of medicine by their feet. Universal adoption of modern medicine based on sound experimental data, toxicity studies and human clinical trials in turn obliterated the use of most of the traditional systems of medicine and the practice of traditional medicine eventually receded more or less to the country side.

The plant kingdom has long served the main resource base for the traditional medicines. This began to change in the 1930's with the advent of synthetic chemistry and was cemented in the 1950s with the introduction of laboratory-bred 'wonder drugs' such as the antibacterial sulfonamides or sulfa drugs. Predictably, the American pharmaceutical industry quickly lost interest in natural products as sources of potential new medicines. When Schultes, the renonwed American Ethnobotanist, returned to Harvard in 1939 after completing ethnobotanical

research among the Indians of southern Mexico, he was unable to find an American drug firm or even a single American chemist willing to work with him. They dismissed him saying they did't want to waste their time looking at these folk medicines. Finally, he has to send his materials to a sympathetic young Swedish chemist for analysis. This chemist, Albert Holfmann, later became famous as the inventor of LSD. Less widely known, however, is that he isolated the hallucinogenic alkaloid psilocybin from mushrooms of Schulte's Mexican collections. From this natural model he synthesized the cardiac betablocker visken, which has improved the quality of life for millions of people with heart problems.

Synthetic drugs like the sulfonamides and diazepam (a sedative) have led some chemists the illusion that synthetic chemistry is the sole future of new drug discovery. Chemists having synthesized a few compounds began to consider themselves to be better chemists than nature. In fact, nature had conceived of diazepam long before the modern-day chemists. Several years ago scientists found that tiny amounts of these drugs occur naturally in wheat and potatoes. Similarly, Dr. Norman Farnsworth of the University of Illinois, a leading figure in natural product chemistry, enjoys pointing out how proud chemists were thirty years ago when they synthesized an antidote for accidental poisoning by organophosphorus insecticides. Later it was found that the substance occurred naturally in electric eels.

Revival of Traditional Medicine

Today we find a renewed interest in traditional medicine. During the past decade, there has been an ever increasing demand especially from the developed countries for more and more plant drugs containing medicinally useful alkaloids, polyphenolics, steroids, glycosider and terpenoid derivatives. The revival of interest in natural drugs, especially derived from plants, started in the last decade, mainly because of the widespread belief that 'green' medicine is healthier than synthetic products. This has led to the rapid spurt of demand for health products like herbal teas, ginseng etc. during the 1980's. Similarly tendency has been towards a general increasing preference in the utilization of natural flavours, dyes, preservatives etc. rather than the less expensive synthetics. A classical example for this trend is clear from the vanilla industry where there is a large demand for the much more expensive natural vanallin isolated from the Vanilla plant. According to a survey conducted by WHO, the uses of medicinal plant remedies are on the increase even in the developed countries especially among younger people. In the industrialized countries, the consumers are seeking visible alternatives to modern medicine with its dangers of over medication or harmful side effects of synthetic drugs. The promotive and preventive medicines widely prevalent in oriental systems, especially the Indian (Ayurveda, Siddha and Unani) and the Chinese systems of Medicine, are finding increasing popularity and acceptance in the developing and developed countries. In a recent report (WHO, 1986) the production of traditional plant

remedies in China was valued at US $ 571 million and the countrywide sales of crude drugs at US $ 1.4 billion annually (Li Chaojin, 1987).

The Resource base of Traditional Medicine

The resource base of all traditional medicines is mainly the plants. Animals, animal products and minerals also find use is most of the traditional medicine. The major classical system of medicine like Ayurveda, Sidha, Unani and Amchi together use about 1200 plants species for medicinal purpose. But the tribals of the India are using over 7500 plant species (AIRCPE Report 1994) for treating a variety of ailments (Fig 1). The medicinal plants used in the classical systems are drawn from different regions of the world where as the medicinal plants used by individual tribal community or village folk practitioners are location specific. The classical system may therefore be considered as a knowledge system developed in urban areas that amalgamated, codified and synthesized the medical wisdom of a wider geographical area.

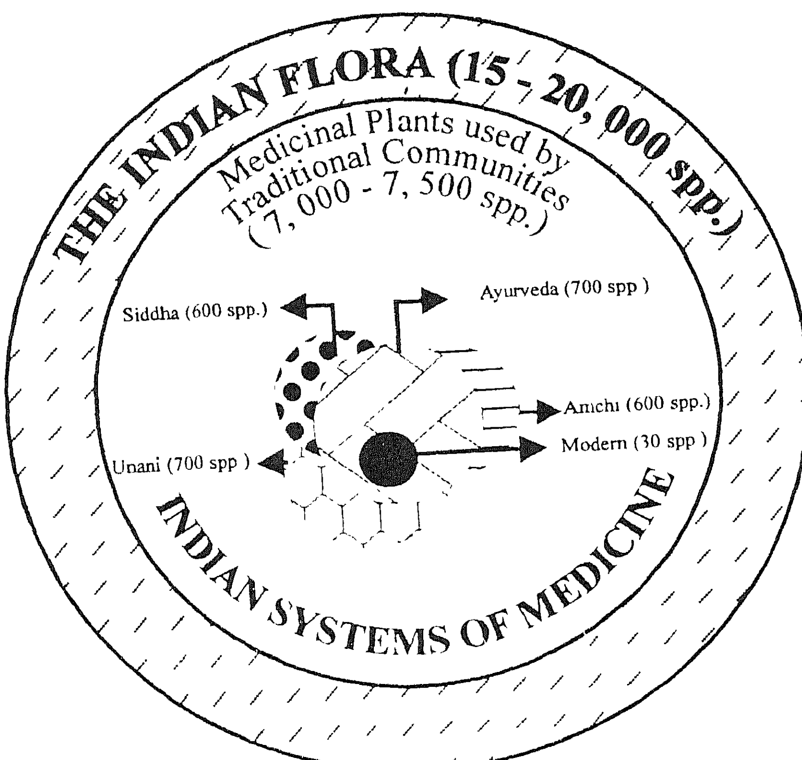

Figure 1 **INDIAN MEDICINAL PLANTS USED IN DIFFERENT SYSTEMS**

Obviously, it is neither possible nor desirable to replace this herbal medicine with western medicine at least during this century. Consequently there is growing interest in medicinal plants. However, with the accelerating destruction of natural resources, particularly in the tropics, it has become clear that the exploitation of medicinal plants must be accompained by conservation measures. Otherwise, these plants become depleted and some of these may even face extinction which will eventually undermine the practice of traditional healers. Over 80% of the medicinal plants required in traditional medicine in the world over are collected from wild sources. The population explosion (a phenomenon of the post industrialized era of today) the improved standard of living led to the ruthless destruction and extraction of the forest wealth including wild medicinal plants leading to the imminent danger of extinction of large number of these plants. The areas that suffer the greatest losses are exactly those of the tropics where not only the medicinal plants are most important to daily health of the local population but also constitute an important component of the diversity of the plant kingdom. Not only do the tropics contain the largest number of species per unit area but the tropical plants also display an amazing variability in their genetic make up e.g., in the biochemical compounds, which they produce (Hamann, 1991). The multitude of ecological interactions among the organisms in the humid tropical forests, for example, produce a vast array of adaptation, many of which are displayed in plants by production of immense number of biochemical compounds. There is urgent need to conserve/preserve these precious plants, many of which are habitat specific, for their sustainable utilization.

Although cultivation of medicinal plants will be the logical route to pursue, in practice this may not always be easy to do. Tropical plants show mostly a very narrow genetic tolerance. Under domestication outside their normal ecological range or habitat or even under disturbed ecosystem, many medicinal plants tend to behave differently. In some cases it becomes difficult to grow them as they may not even survive. In certain cases the plants may survive outside their habitats but may not produce the desired chemical traits i.e., the active principles. Such scenario calls for a different approach in the propagation of plant species of interest. In short, both *in situ* and *ex situ* conservation methods are essential for the conservation of medicinal plants. Botanical gardens, tropical as well as extratropical should play a key role in this direction. Particular emphasis should also be given to *in vitro* preservation i.e., conservation in the form of tissue or shoot cultures which should be kept in existance *ad infinitum*. Cryopreservation of embryos, tissues or pollens is also advisable.

WHO Approach

In the last decade WHO's Health Assembly passed many resolutions in response to the resurgence of interest in the study and use of traditional medicine in health care and in recognition of the importance of plants used by the system. In 1978

WHO called for a comprehensive approach to the subject of medicinal plants (resolution WHA 331:33). This includes:

(1) An inventory and therapeutic classification of medicinal plants used in different countries.

(2) Scientific criteria and methods for assessing the safety of medicinal plant products and their efficacy in the treatment of specific conditions and diseases.

(3) International standards and specifications for identity, purity, strength and manufacturing practices of medicinal plant products.

(4) Methods for safe and effective use of medicinal plant products by various levels of health workers. Dissemination of such information among member states and designation of research and training centres for the study of medicinal plants.

In May, 1987 the 40th WHA resolution (WHA 40:33) reaffirmed the earlier resolutions and provided a mandate for future action in this field. The member states were urged:

(1) To initiate comprehensive programmes for the identification, evaluation, preparation, cultivation and conservation of medicinal plants used in traditional medicine.

(2) To ensure quality control of drugs derived from traditional plant remedies by using modern techniques and applying suitable standards and good manufacturing practices.

China – An Example to Simulate

China is perhaps the only country in the world who has demonstrated the best use of traditional medicine in providing the best health care to her people. In 1944, the Chinese communist called to reform the traditional doctors so as to make one strong front towards common public health goals. In the National Health Conference held in 1950, China laid down the guiding principle for the national health policy which included:

1. Health care must serve the common people.
2. Priority must be given to the prevention of disease with prophylaxis and treatment combined.
3. Health compaign should be conducted together with other mass compaign.
4. Western and traditional medicine should work together and eventually get integrated.
5. Chinese medicine improvement school and united clinics were started by the Government, in which traditional and modern doctors could establish team work.

Traditional doctors entered government health units and modern hospitals. The modern medicine graduates in 1950s were given 3 extra years teaching and training in traditional Chinese medicine and they constituted the initial intellectual body for the promotion and integration of modern medicine and traditional medicine in

China. The modern medicine in China is now essentially becoming an extension of the local traditional medicine. The barefoot doctor is just not barefoot, he is a peasant who performs medicinal duties after being locally trained.

Chain has eventually made its traditional medicine as an integral part of the formal health care system and utilizes in about 40% of cases at the primary care level. Supplies are assured by the State-owned Chinese Crude Drug company which has branches in all provinces. Formerly crude drugs were mostly collected in the wild state but as natural sources started becoming depleted more plant species were brought under cultivation. Special encouragement has, therefore, been given for the cultivation of medicinal plants. Agriculture departments at all levels took part in formulating policy and establishing plantations which covered some 330,000 hectares. (Lichaojin, 1987). They have scientifically validated and upgraded the technology of production and administration of a large number of traditional herbal drugs. China has currently 882 herbal drugs in use. Chinese Academy of Medicinal Science, Peking, issued the National Pharmacopoeia in two volumes—perhaps the only modern pharmacopoeia of the world that gives full credit and recognition to traditional medicine. Chinese medicine employs 4941 species of vascular plants belonging to 1544 genera and 240 families out of 35,000 species of vascular plants belonging to 2949 genera and 287 families reported from China.

Research Needs in Traditional Medicine

From what is already known about the richness of the traditional medicine including that of the tribals, it is going to be perhaps the richest resource base for the health care of humankind. Not only it is a natural source for healing the ailstricken humanity at the cheapest cost, but also provides effective drugs without any side effects satisfying the socio-cultural ethos for the community.

The intrinsic value of traditional medicine in imparting health care to people, particularly to the rural mass of the developing countries is now well recognised. The therapeutic value of many traditional remedies is also no longer open to doubt. But the common notion that all remedies of natural origin are harmless and carry no risk to the consumer is not fully true. Many plants and animal products and minerals used in many traditional remedies are highly toxic. It is true that there are methods prescribed in certain traditional systems like Ayurveda, Siddha, Unani etc. for detoxifying such toxic products. But there are instances where such preparations are found to be very toxic. In such cases proof of safety should take precedence over establishing efficacy. Health authorities are required to exercise close surveillance in such cases. It is also desirable to scientifically evaluate the traditional remedies particularly those lesser known tribal remedies, to ensure safety, efficacy, stability, standards and dosage formulations etc. Efforts should also be made to determine the composition of the therapeutic principle. Correct labelling of the constitutents of herbal remedies is critical for safety evaluation and quality control.

Scientific validation and fixing standards for simple particularly single plant based remedies are comparatively easy. But such evaluations may pose problems with compound formulation containing several ingredients like those of the organised systems like Ayurveda, Siddha, Unani and Amchi. The therapeutic effect of the formulations with multiple ingredients are possibly due to compound effect or synergistic effect of a number of compounds. It is also possible that the active compounds when isolated in pure form although active may be very toxic But in the natural form it is in association with other compounds either derived from the same plant or other plants or other materials in the formulation the toxic effect is either minimised or become absolutely non-toxic. This whole subject of the rationale for compound prescription of medicinal plants and the herbo-mineral combinations offers a vast field of research.

Research in ethnomedicine should be directed towards finding alternative methods to replace (i) the unrealistic L.D. 50 test to test the toxicity of drugs and (ii) the efficacy evaluation by solvent extraction and feeding to the animals to see the activity in them. Also the classical pharmacological approach to study the herbal drugs by isolation of active principle(s) — single compounds — their structure elucidation followed by testing their biological activity etc. are far from satisfactory. The evaluation of traditional medicine requires an integrated approach that combines the concepts and theoretical foundations, particularly of the classical traditions like Ayurveda, Siddha or Unani with the modern scientific knowledge, technology and tools. The concept of ethnopharmacology is evolved out of such a requirement. In India the ethnopharmacological approach to evaluate the traditional medicine was initiated at Regional Research Laboratory, Jammu in 1987 when this author was working at that centre. Now, a full fledged ethnopharmacology laboratory is functioning at Tropical Botanic Garden and Research Institute (TBGRI) Palode, Kerala. The ethnopharmacology division of TBGRI has a team of scientists drawn from Ayurveda, tribal medicine, ethnobiology, phytochemistry, biochemistry, modern medicine, pharmacology and pharmacy etc. The objective is to develop appropriate techniques to study the traditional medicine in line with the classical concepts of pharmacy and pharmacology — like the Rasa, Guna, Veerya, Vipaka, Prabhava concept of Ayurveda etc. and blend it with that of the scientific knowledge of modern medicine and apply the tools and technology to evaluate and formulate and prepare herbal remedies. The results are encouraging. TBGRI team is successful in developing various new herbal formulations and some of which are now at advanced stage of clinical evaluation and will be released for commercial production shortly. Concerted efforts in this line of research is essential in updating the traditional medicine and make it competent to effectively meet the health care needs of the people of the Third World and thereby achieve the WHO goal of Helath for All By 2000 AD. Not only it is a natural source for healing the ail stricken humanity at the cheapest cost, but also provides effective drugs without any side effects and satisfying the socio-cultural ethos of the community.

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Nomenclatural Ambiguity of Medicinal Plants used in Indigenous Systems of Medicine

M.P. Sharma

Department of Botany, Hamdard University
Hamdard Nagar, New Delhi 110062

MEDICINAL plants have been used for the treatment of human ailments and suffering since time immemorial. In India, the knowledge of herbal medicine is widespread - ranging from tribal folklore use to age-old practices and closely guarded recipes handed down from generation to generation, to highly evolved systems of medicine like Ayurveda, Siddha and Unani-Tibb. Ayurveda and Siddha have been part of Indian civilization since its inception. The Unani system of medicine was introduced in this country in the 10th century A.D. by Muslim rulers. Later on a number of Ayurvedic plants were adopted by Unani physicians and vice-versa. These systems have served mankind through the centuries and it is certain that for indefinite period more they will continue to be in use.

Of the 20,000 and odd species of plants reported to be growing in India, about 2,500 species have therapeutic value (Chopra *et al.*, 1956) and more than 500 are used in indigenous systems of medicine. A large number of plants listed in pharmacopoeias are not being utilized by the drug industries in the traditional systems of medicine due to lack of correct identity, standardization and quality control of plant material forming the drug on which these systems depend heavily. The lack of exposure of later day physicians and researchers to science of Botany and Pharmacognosy, made them dependent on herb collectors which consequently caused deterioration in the efficacy of preparations. In ancient time, people,

particularly the physicians had no problem in identifying medicinal plants as they had very close contact with nature and were able to identify plants whose roots, leaves, bark, flower, fruits and seeds were useful in the preparation of medicines. But gradually in the process of urbanization this contact with nature was cut off and consequently the knowledge about identification of plants also deteriorated to a great extent. The subsequent practitioners of these traditional systems tried to identify drug plants on the basis of morphological characters mentioned in the old treatise of these systems which has resulted in coining of the same name to more than one plant or different names applied to one plant. This has put many plants in the controversial position, and their identity has become doubtful. It can be seen in the available literature on Indian medicinal plants (Anonymous 1948-1976; Bal 1932; Kirtikar & Basu, 1935; Nadkarni, 1954; Chopra *et al.*,1956, 1958, 1959; Jain, 1968; Satyavati *et al.*, 1976; Dey, 1980; Satyavati & Seth, 1987; Mitra, 1985) that in many cases quite unrelated plants are given the same name or same plant is given different names. Due to the ambiguity of plant names overlapping of data may occur in chemical or pharmacological informations where the botanical identity of market sample of crude drug taken up for research is not ascertained. The botanical identity of many such controversial plants is yet to be resolved (Wahid & Siddiqui, 1961; Bapalal, 1974, 1975; Ramachandran & Mehtani, 1990).In the present communication an attempt has been made to compile a list of such plants which are known in the literature or are sold in market by different names and vice- versa. The plants are enumerated alphabatically. Synonym has been avoided for the sake of economy of space. Only Sanskrit, Unani, common or trade name of the plants are given. Local names are not given, since they vary from locality to locality due to the existence of several regional languages and innumerable dilects in India.

I. Sanskrit, Unani, Common or Trade Names applied to more than One Plant

Agnimantha - *Clerodendrum phlomides* L.f.; *Premna obtusifolia* R.Br.

Ajmuda -*Trachyspermum roxburghianum* (DC.) Craib; *Apium graveolens* L.

Amlevetta - *Rumex vesicarius* L.; *Garcinia pedunculata* Roxb. Ar(a)lu - *Oroxylum indicum* (L.) Vent.; *Orchis latifolia* L. Ar(a)ni - *Clerodendrum phlomides* L.f.; *Premna obtusifolia* R.Br.

Ashoka - *Saraca asoca* (Roxb.) De Wilde; *Polyalthia longifolia* Thw.

Badaward - *Cardus nutans* L.; *Fagonia arabica* L.; *Volutarella ramosa* (Roxb.) Santapau.

Bak(am) - *Sesbania grandiflora* Pers; *Caesalpinia sappan* L.

Bala - *Sida cordifolia* L.; *S. acuta* Burm. f.; *Pavonia odorata* Willd.

B(a)rahikand - *Tacca integrifolia* Ker-Gawl.; *Dioscorea* spp.

Bhumikushmanda - *Pueraria tuberosa* DC.;*Ipomoea paniculata* R.Br.

Bidarikand - *Ipomoea digitala* L.; *Pueraria tuberosa* DC.

Brahmdandi - *Echinops echinata* DC.; *Argemone mexicana* L.; *Tricholepis glaberrima* DC.

Brahmi - *Bacopa monniera* (L.) Pennell; *Centella asiatica* (L.) Urban

Celery - *Apium graveolens* L.; *Trachyspermum roxburghianum* (DC.) Craib.

Cha(u)lmoogra - *Gynocardia odorata* R.Br.; *Hydnocarpus laurifolia* (Denn.) Sleum.

Devdar(u) - *Cedrus deodara* (Roxb. ex Lamb.) G.Don.; *Polyalthea longifolia* Thw.

Dicamali - *Gardenia lucida* Roxb.; *Peucedanum grande* Cl.

Gaojaban, Gajawan, Gaozaban, Gayuban - *Caccinea macranthera* Brand. var. *crassifolia; Onosma bracteatum* Wall.

Gokharu, Goksh(u)ru, Gokshura(k) - Tribulus terrestris L.; *Pedalium murex* L.

Indian Gentian - *Gentiana kurroo* Royle; *Picrorhiza kurroa* Royle ex Benth.

Kadu - *Gentiana kurroo* Royle; *Picrorhiza kurroa* Royle ex Benth.

Kahu(a) - *Lactuca sativa* L.; *Terminalia arjuna* (Roxb.) Wt. & Arn.

Kak(a) Jangha - *Asteracantha longifolia* Nees; *Leea acquata* L.

Kakoli - *Lillium polyphyllum* Don; *Luvunga scandens* (Roxb.) Buch. - Ham. ex Wight

Kalijri - *Vernonia anthelmintica* Willd.; *Nigella sativa* L.

Kanranj(a)Bij - *Capparis spinosa* L.; *Pongamia pinnata* Pierre.

Khadir (Chhal) - Acacia catechu Willd.; *Uncaria gambier* Roxb.

Khetpapra - *Oldenlandia aspera* DC.; *Rungia repens* Nees.

Kukti - *Gentiana kurroo* Royle; *Picrorhiza kurroa* Royle ex Benth.

Mari - *Caryota urens* L.; *Piper nigrum* L.

Mayur shikha - *Celosia argentea* L.; *Actinopteris dichotoma* Bedd.; *Adiantum caudatum* L.

Meda - *Laptadaenia reticulata* Wt. & Arn.; *Litsea monopetala* (Roxb.) Pers.

Moth(a) - *Cyperus rotundus* L.; *Phaseolus aconitifolius* Jacq.

Murva - *Clematis triloba* Heyne ex Roth; *Origanum majorina* L.

Pashanbhed - *Bergenia ligulata* (Wall.) Engl.; *Coleus aromaticus* Benth.; *Didymocarpus pedicellata* R.Br.

Pitpapada - *Fumaria officinalis* L.; *F.indica* (Hsussk.) Pugsley; *Hedyotis corymbosa* (L.) Lamk.; *Peristrophe bicalyeulata* (Retz.) Nees.

Priyangu - *Aglaia roxburghiana* Mia.; *Prunus mahaleb* L.

Ras(h)na - *Alpinia officinarum Hance; Pluchea lanceolata* Oliver; *Vanda tessellata* (Roxb.) Hook. ex G. Don.

Ratanjot - *Geranium wallichianum* sw.; *Onosma echioides* L.; *Clausena pentaphylla* D.C. *Potentilla nepalenses* Hook.

Rudanti - *Capparis moonii* Wight.; *Cressa cretica* L.

Salam (misri) (panja) - *Eulophia campestris* Wall.; *Orchis habenarioides* King & Prantl

Sankhpushpi - *Canscora decussata* Schult.; *Convolvulus microphyllus* Sieb. ex Spreng; *Evolvulus alsinoides* L.; *Clitoria ternatea* L.

Satmuli - *Asparagus racemosus* Willd.; *Emilia sonchifolia* DC.

Somlata - *Ephedra gerardiana* Wall.; *Sarcostema acidum* (Roxb.) Voigt.

Talispatra - *Abies webbiana* Lindl.; *Taxus baccata* L.; *Rhododendron anthopogon* D.Don.

II. Different Names (including Sanskrit, Unani, Common or Trade Names) applied to the same Plant

Abroma augusta L.f. - Abroma, Olat-Kambal, Ulat-kambal

Abrus precatorius L. - Gumchi, Gunja, Kanchabeej, Ratti

Abutilon indicum G. Don - Atibala, Kanghi, Kamkatimoola

Achyranthes aspera L. - Apamarga, Apang, Chirchira

Aconitum heterophyllum Wall. - Atis, Ativisha, Shringi, Shrigik

Acorus calamus L. - Bach, Boch, Buch, Calamus, Vacha

Adhatoda zeylanica Medik - Arusa, Vasa(ka)

Alhagi pseudalhagi (Bieb.) Desv. - Durlava, Duralabha, Javasa

Aloe barbedensis Mell. -Aloes, Eliyak, Elua, Elva, Gheekuar, Ghikanwal, Ghrita Kumari, Kumari, Kuvarpatha, Musabbar.

Alstonia scholasis (L.) R.Br. - Chatwan, Chhatim, Dita, Saptaparna

Anethum sowa Roxb. - Anethi, Anithi, Satapushpi, Soia, Sowa, Soyak

Anogeissus latifolia (DC.) Wall. ex Bedd. - Dhataki, Dhava Dhavdiphool

Apium graveolens L. - Ajmod(a), Ajmuda, Randhuni, Chanu.

Argemone mexicana L. - Piladhatoora, Satyanasi, Swarnakshiri

Argyreia nervosa (Burm.f.)Boj. - Samudrapalaka, Bidhara, Samudrasosh(Beej), Vridha daruk, Vrikshamla, Vrudhadaruk, Vrudhhadaur

Artemisia maritima L - Kirmala, Shih, Kirmani

Asparagus racemosus Willd. - Satumuli, Satamuli, Satamundi, Shatavari, Satavar(y), Shaqakul-Misri

Bacopa monniera (L.) Pennell - Brahmi, Jalbrahmi

Bambusa arundnacea (Retz.) Roxb. - Vans(h)a, Bansalochan, Bamboo manna, Vans(h)alochan

Berberis aristata DC. - Daruhald(i), Dur(u)hald(e), Rasot, Daruharidra, Darvi, Rasanjan, Berberis, Rasavauti, Rasaunt

Bergenia ligulata (Wall.) Engl. Pashanbhed, Patharchati

Bombax ceiba L. - Semal, Shalmali(mool), Musli Senbhal, Mochras *Butea monosperma* (Lam.) Taub. - Palashushpa, Palas(bij), Kamarkas

Caccinia glauca Savi - Gaozaban, Gajawan, Gayuban

Caesalpinia bonduc (L.) Roxb. emend. Dandy & Excell - Gajga, Kakachia, Karanj, Kuberex, Sagargota, Nata Karanj, Putikaranj

Calotropis gigantea (L.)R.Br. - Ark, Aak, Akanda, Madar, Calotropis

Capparis moonii Wight - Wagati, Rudanti, Luthikai

Carum carvi L. - Jira siyah, Shiajira, Jira, Krishana Jirak, Sankhajiru, Caraway

Cassia angustifolia Vahl - Senna (pod), Sonamukhidoda, Markandi, Sonamukhi, Swarnapartri, Cassia

Cassia fistula L. - Amaltas, Aragdah, Aragvadha, Aragwadi, Cassia, Germala, Garmalo

Cassia occidentalis L. - Kasari, Kasaundi, Kasondi, As(h) and (a)ra.

Cedrus deodara (Roxb. ex Lamb.) G.Don - Deodar, Devdaru, Cedarwood

Centella asiatica (L.) Urban - Brahmi, Mandukparni, Centella, Tholkuri, Thankuni

Cinnamomum tamala Nees ex. Eberm. - Tamalpatra, Tejpat(tar), Tuj, Twak

Cinnamomum zeylanicum Breyn - Darchini, Cinnam, Cinnaman(i)

Cissampelos pareira L. - Kalipat(h), Pahadmool, Katori, Akanadi

Clerodendrum phlomides L.f - Bharangi, Agnimantha, Ar(a)ni

Crocus sativus L.- Keshar, Saffron, Zafran, Kumkum

Cuminum cyminum L.- Jirak, Jeera, Cumin, Jeevaka, Jira safed, Shwet Jirak.

Curcuma longa L. - Haridra, Cancha haridra, Haldi, Gandhapalasi, Kancha haridra

Cyperus rotundus L. - Nagarmotha, Moth(a) Must(h)a, Mustak, Nut Grass

Didymocarpus pedicellata R.Br. - Patharthodi, Patharphodi, Patharphori, Pashanbhed

Eclipta alba (L.) Hassk. - Bhangra, Bharan(a) raja, Bhringaraja

Elettaria cardamomum (L.) Maton - Belchi, Ela(ichi), Ellaichi Khurd, Kagdi Elaichi, Welchi, Sukshma Ela, Cardam(om), Choti Elaichi

Embelia ribes Burm. - Biranga, Vavding, Vidang(a), Babring, Bidang, Wavding

Enicostema hyssopifolium (Willd.) Verdoorn - Mamajjak, Mamajawa, Chhota Chirayta

Eulophia campestris Wall. - Mujjatak, Salib Shalabmisri, Salam (panja)

Euphorbia neriifolia L. - Manasa Sij, Euphorbia, Snuhi (Dugdha), Sahund

Evolvulus alsinoides L. - Kali-sankhavli, Sankhapushpi, Vishnugandhi

Ficus racemosa L. - Gular, Fig, Udambar, Udumber, Umbra (Paan (Phal)

Foeniculam vulgare Mill. - Saunf, Sounf, S(h)atapushpa, Madhurika Mouri, Fennel, Foeniculam

Gentiana kurroo Royle - Kadu, Karu, Gentian, Indian Gentian

Glycyrrhiza glabra L. - Liquorice, Glycyrhizal, Glycerhiza, Mulethi, Jetimadh, Licorice, Madhueshti, Jyeshthimadh, Yashtimadhu, Yashti, Jastimadhu, Asi-us-soos

Gmelina arborea Roxb. - Gambhar(i), Kashmari

Gynocardia odorata R.Br.- Bandrephal, Chau(u)lmoogra

Hemidesmus indicus (L.) Schult. - Indian Sarsaparilla, Anantamool(a) Salsa, Hemidesmus

Hibiscus rosasinensis L. - Jaba, Jawakusum, Gudhal, Gul-gudhal

Holarrhena antidysenterica (L.) Wall. ex. DC. - Kutaja, Kadachhal, Kudachhal, Indrajava, Indrayava, Kurchi

Hydnocarpus laurifolia (Denn.) Sleum. - Tu(r)varaka, Cha(u)lmoogra, Hydnocarpus

Hygrophila auriculata (Schum.) Heine - Kokilakshya, Asteracantha, Kulekhara, Kuliakhara, Kakjangha, Gokkasu

Ipomoea mauritiana Jocq. - Bhumikushmanda, Bhuikobola, Bhuipatri, Bidarikanda, Bidhari, Vidarikand

Ipomoea nil (L.) Roth - Krishna til, Kaladana, Krishna beej

Lepidium sativum L. Asalio, Halim, Ashelia, Chandra, Chandrika

Leptadaenia reticulata (Retz.) Wt. & Arn. - Jivanti, Meda, Dodi, Jeevant(h)i, Dronapushpi

Luffa acutangula (L.) Roxb. - Luffa, Koshataki, Shirola, Turai

Melia azedarach L. - Mahanimb, Mahaneem, Bakayan

Mesua ferrea L. - Nages(h)war, Nagkes(h)ar, Nagpus(h)p(y)a

Mimusops elengi L. - Bakul, Maulsari, Molsari

Moringa oleifera Lam. - Sajina, Sigru, Shigru (twak) Saragva (Bij), Shajan, Moringa, Saragwa (Chhal)

Mucuna prurita Hook. - Banari, Kapicachhu, Benarmul, Kaucha, Kauncha, Alkushi, Atmagupta

Myristica fragrance Hoult. - Nutmeg, Mace, Jaiphal, Jayaphal, Jat(i)patri, Jatiphalam, Javitri, Javantri, Javintiri

Nardostachys grandiflora DC. - Jantamansi, Balchad, Balchar, Mansi, Masi

Nigella sativa L. - Kalaunji, Jira, Kalijiri, Kalonji, Black cuminum, Upakunchika

Onosma bracteatum Wall. - Gajawan, Gaozaban, Gojihivat, Gojiwha, Gayuban

Operculina turpethum (L.) Silva-Manso - Turpethum, Trivrit, Truvrit, Nishoth, Nishottar, Trivrut(a).

Orchis habernariodes King and Prantl - Panjasalam, Salam (misri) (panja)

Orchis latifolia L. - Aralu, Salep, Salap

Oroxylum indicum (L.) Vent. - Shyonak, Ar(a)lu, Ardusi, Sheonak

Pavonia odorata Willd. - Netravala, Netrabala, Herber, Sugandhbala, Netarmool

Pedalium murex L. - Gokshura(k), Gokhru Mota, Mota Gokhru, Goksh(u)ru

Picrorhiza kurroa Royle ex Benth. - Kat(u)ki, Kadu, Kutki, Balkadu, Picroh, Kalikutki

Pimpenella anisum L. - Badyan, Badian, Anise, Aniseed, Anisi, Saunf, Sounf

Piper cubeba L. f. - Kankol, Shitalchini, Kabachini, Cubeb, Chankabad, Chini Kabab

Paper longum L. - Long piper, Pipal, Pipul, Pipli(mul), Long pepper, Piper, Pippalimul

Pipur nigrum L. - Kalimiri, Maricha, Kalimirchi, Black Piper, Mirch Seah, Piper, White Marich, Mari

Polyalthia longifolia Thw. - As(h)ok(a), Devdaru, Debdaru

Premna obtusifolia R.Br. - Karnika, Agetha, Agnimantha, Ganiyar

Pterocarpus marsupium Roxb. - Asan, Vijayasar, Hirdakhan, Bijesar

Pueraria tuberosa DC - Vidarikanda, Bidarikand, Bhumikushmanda, Bidhari

Rheum emodii Wall.ex Meissn. - Rhubarb, Revandchini, Amlaparni, Ravanchi, Shiro, Revanchu, Lakdu, Rewandchini

Rubia cordifolia L. - Manjistha, Majith, Manjit, Manjistha

Rungia repens (L.) Nees - Kharmor, Khetpapra, Parpat

Saccharum spontaneum Linn.- Kans, Kash, Kansda mool, Sherdi (mool)

Saussurea lappa Clarke - Kusth(a), Kur, Kusta, Saussurea, Uplet, Kut

Semecarpus anacardium L.f. - Bhallatak(a), Bhilawa, Bhela

Sesbania grandiflora (L.) Poir - Agastya, Bak, Rakta Agastya, Agati, Bakam

Sida cordifolia L. - Balapanchanga, Bala, Bare(l)la, Berula, Bariar(a), Khareti, Kharenti, Bariyer

Similex china L. - Chob-Chini, China, Chopchini, Topchini

Solanum indicum L. - Brihati (Kar), Vrihati, Brah(e)ti, Kateri, Brah(u)ti

S. surattense Burm. f. - Kantikari, Kantakari(ka), Kateli, Bhuiringani, Brihati(kar), Brah(u)ti, Brah(e)ti, Kater

Sphaeranthus indicus L. -Munditika, Mundi, Gorak(h)mundi

Sterospermum suaveolens DC. - Patha, Tamarapushpa, Pat(a)la

Strychnos nux-vomica L. - Kuch, Kuch(i)la, Kuchelaka, Nux-vomica, Vishatinduk, Vishamusti

Swertia chirayata (Roxb. ex Flem.) — Karst. Kirata(k), Kirayat, Kirayta, Kariyetu, Chireta, Chiraita, Bhunimba, Lodhra, Lodh(ar)

Syzygium cuminii (L.) Skeels - Jaman, Jamun Jambool, Jambu(bij)

S. aromaticum (L.) Merrill et Perry - Lavang(a), Laung, Caryoph, Qaranphal, Mehaka, Clove, Qaranfal

Tephrosia purpurea (L.) Pers. - Sharpankha, Sarapunkha, Shripankha

Terminalia arjuna (Roxb.) Wt. & Arn. - Arjan(a), Kahu(a), Koh

T. bellirica (Gaertn.) Roxb. - Bibhitak, Bahera, Behda

T. chebula Retz. - Shiva, Putina, Harde, Hararrh, Haritaki, Harre, Pilihar(i) Pilher Abhaya, Himaj, Harar(a), Bal Harde

Tinospora cordifolia (Willd.) Hook. f. & Thoms. - Gulvel, Guduchi, Guddochi, Giloe, Guducha, Gaduchi, Gulancha, Guruchi, Giloy, Amruta, Amrita

Trachyspermum ammi (L.) Sprague - Aj(o)wain, Ajovan, Ajma, Ptychotis

Trachyspermum roxburghianum (DC.) Craib - Ajmood(a), Ajmuda, Celery, Yavan(i), Curcuma Ajmoda, Rajani

Uraria lagopodioides (L.) Desv. ex DC.- Prishnaparni, Prasniparni

Urginea indica (Roxb.) Kunth - Urginea, Squill, Parnabija, Jangli Pyej, Indian squill, Coromandel Squill

Urgenia maritima Babor - Scilla(e), White squill, European Squil

Vateria indica L. — Ral, Kaherba, Pisthi, Sarj, Swarjika

Veteveria zizanioides (L.) Nash - Khas, Valak, Usheeram, Ushir, Usir

Viburnum prunifolium L. - Viburni, Viburni pruni (fol), Black Haw, Viburnum Pruni, Viburnum

Zingiber officinale Rosh. - Sunth(i), Adrak, A(rd)arak, Ginger, Indian Ginger, Pulvginger, Sonth, Zanjebeel Zinger, Zingiberis

In the recent years, a great emphasis is being given to analyse the drugs used in traditional systems of medicine for various ailments. Majority of the pharmaceutical industries, herbal practitioners and common people depend largely upon crude drug dealers for the supply of plant materials. These plants are generally collected by the professional collection who may not be a botanist or taxonomist. Sometimes the identity of crude drug purchased from market, which is based on trade or

vernacular name, is taken for granted without subjecting the plant material for stringent method of botanical identification. It is equally possible that one may not get the desired plant as the trader may supply other plant having same trade or vernacular name. In addition to nomenclatural ambiguity, bazar drugs are adulterated, sophisticated or substituted by quite unrelated plant materials. Since there is no government control over the sale of adulterated or spurious drugs, the traders take full advantage of this and sell to their customer anything that resemble or may be made to resemble the genuine drug. Thus, authentication of botanical source of plant material taken up for research or medicinal use is necessary to achieve satisfactory results and also to maintain efficacy and therapeutic property of the preparation in which these plants are used.

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Medicinal Plants of Bhutan

Sadruddin

Department of Botany
Sherubtse College, Kanglung
Bhutan

Introduction

PLANTS undoubtedly play a major role in the economic, social and cultural life of human beings especially because of their uses as food, clothings, drugs and medicines etc. (Ahmad, 1992; Gangwar, 1987; and George *et al.*, 1985). Traditional therapy is often believed to be more efficacious than the modern system except in case of sophisticated surgery Since there are no established side effects of the traditional remedies, the ethnomedicinal system is lately attracting a larger number of people to its fold.

A considerable number of people in Bhutan prefer to utilize plant resources for their health care and maintenance. In present times, medicinal plants are attracting much higher attention all over the World but needless to emphasize that ethnomedicinal studies are a prerequisite for all the pharmacological, pharmacognostical and clinical researches. Realizing their importance, the Royal Government of Bhutan has made appreciable efforts to preserve and perpetuate the indigenous knowledge of medicinal plants by establishing indigenous medicine hospitals in different parts of the country under the control of the department of Medicinal plants. However, there are some knowledgeable persons in villages who are regarded as the masters of this great herbal treasure. This paper is, therefore, an attempt to document this ethnomedicinal knowledge of plants of Bhutan.

Bhutan, the land of Peaceful Dragon, was in the past called "Sman Lzongs Rgyal Khab", meaning the land of medicinal plants (Dorji & Morisco, 1989).

Covering an area of 41000 sq. kms, extending from 88° 45° to 92° 10° east and from 26° 40° to 28° 21° north at an altitude of 150-7554 m., the country is situated on the eastern Himalayas, bounded by the Indian states : Arunachal in east, Assam and W. Bengal in South and Sikkim in West; in the north it faces Tibet (China).

The climate of Bhutan is so diverse and enchanting that Chatterjee (1965) has vividly described the rapid climatic changes: "The intense cold of Siberian Winter, the terrific heat of the Sahara desert and the mild pleasant weather of mediterranean Italy may be experienced in the course of a single days journey in Bhutan ". Under such congenial environmental conditions Bhutan has been endowed with luxuriant vegetation of tropical, subtropical, temperate and alpine types.

Botanically Bhutan was hardly known to the World until very recently when the Flora of Bhutan was published (Grierson and Long, 1983). Among all the exploratory trips conducted by different botanists and explorers, only a few expeditions have been undertaken to Bhutan which were purely for Botanical and floristic studies (Hara, 1966, 1971; Subramanyam, 1973, Grierson & Long, 1983 and Sadruddin, 1993). But not much effort has been made to study the medicinal plants of Bhutan. Hence, this is an attempt to enlist the medicinal plants of Bhutan and document their uses by the local people, supplemented with the data as reported in the standard literature.

The present paper enlists 15 important wild plants of medicinal values of Bhutan. Botanical names, local names and distribution in Bhutan have been given. The medicinal values based on the local knowledge existing with the people and as reported in the standard literature have been presented. It is feared that due to the progressive modernization and ignorance also this great herbal treasure may vanish in due course of time. Therefore, the preservation of such herbal wealth and their values is highly recommended.

In the following enumeration only important plants are mentioned and briefly described. The species are arranged alphabetically. Account of distribution of each species has been restricted to Bhutan only. Botanical names of each species are followed by their families and local names in respective dialect, which is indicated in bracket. Abbreviation Dz stands for Dzongkha, the national language of Bhutan, Sh for sharshope, Nep for Nepali, a dialect commonly spoken mostly by the inhabitants of south Bhutan, and Eng for English name.

1. *Achyranthes aspera* Linn.

Amaranthaceae, The Prickly Chaff Flowers (Eng).

Erect or sprawling herb with long branches 25-100 cm. Leaves elliptic 4 - 11 × 1.5 - 6 cm, acute, base cuneate, sparsely to oppressed, pubescent. Flowers small 3 - 5 mm on long spike upto 25 cm greenish, bracts spinous. Fruits an oblong sticky utricle 2m.

Fl.& Fr. : June - Oct.

Altitude : 300 - 1450 m.

Distribution : Phuntsholing, Deothang, Punakha, Mongar and Trashigang Districts.

Uses: The plant is medicinally important. The economically important part is the root. Local people dig out fresh roots and crush in little water. The juice, obtained in this way, is given to cure preliminary stage of pneumonia with satisfactory result.

In wealth of India also the medicinal value of this plant has been highlighted. The flowering spikes or seeds, ground into a paste, are used as an external application for poisonous insect bites.

A decoction of the entire plant is reported to possess diuretic properties and occasionally it has proved useful in renal dropsies.

Studies done by Gupta (1981) from Bihar brings out interesting medicinal value. He has reported that the root of this shrub are pounded and boiled in water. The decoction is administered to women after child birth. In Almora hills, infusion of leaves of *Achyranthes aspera* (Chirchira) in alcohol is used for controlling and curing leucoderma (Gupta, 1981).

Jain (1981) reported that root bark of *Achyranthes aspera, Solanum torvum* and the bark of *Santalum album* are made into pills. The pills are administered three times a day with hot water for malaria. Manandhar (1989) has reported the plant as diuretic and its ash is used in dental problems. In India the herb is used in cases of dropsy while the leaf juice is applied to the stings of scorpion. Maiden (1989) mentioned that the seed paste is given for hydrophobia and in cases of snakebite.

The infusion of the root is astringent, and its paste is taken to treat stomach troubles. In Assam, the inhabitants take its seeds with milk to check hunger without any loss of body weight, and the roots are tied to the hair of pregnant women before delivery to ease the birth (Majumdar *et al.* 1978). Yonzone *et al.* (1984) reported that in Darjeeling the plant is used to treat piles and rheumatism.

2. *Betula utilis* Don.

Betulaceae, La Tap (Dz), Bhojpatra (Nep) and Himalayan paper birch (Eng).

A deciduous tree, bark smooth, peeling in papery sheets. Leaves stalked, ovate, long pointed, sharply and irregularly toothed, 3 - 12 × 2 - 8 cm. Male catkins drooping, solitary, female spike solitary, erect.

Fl. & Fr. : April - June

Altitude : 3000 - 4200 m.

Distribution : Thimphu, Tongsa, Bumthang and Trashigang, upper Mochu and Kulong chu.

Uses : The bark smells like tooth pastes and therefore tender branches are known to be used as tooth brush for cleaning teeth. Manandhar (1989) reported that an infusion of the bark is carminative and is also prescribed for hysteria. It has antiseptic and aromatic properties. The paste of the bark is used in cases of jaundice and earache. Shah *et al.*(1971) reported that the resin is used as contraceptive in Kumaon.

3. *Bergenia ciliata* (Haw.) Sternb

Saxifragaceae, Pakhanbed (Nep) and Rock foil (Eng).

A herb with thick root stock, leaves stalked, 3.5 - 16.5 × 3 - 12 cm, suborbicular, entire, fringed with short stiff hairs.

Flowers pink or pink purple

Fl.& Fr. : February - May

Altitude : 1500 - 3050 m.

Distribution : Phuntsholing, Deothang, Haa, Mongar districts and Kanglung.

Uses: The plant is very useful medicinally. The powder of rhizome is used to cure stomach problems and fever. Manandhar (1989) has reported that besides other ailments, the plant is used in urinary troubles.

The rhizome is used to cure piles, urinary troubles and asthma. The squeezed rhizome is boiled and the decoction is taken to treat gout. Its paste is applied to boils and said to be effective for backache. Dobremez (1976) mentions that it is good for diarrhoea and fever. The rhizome contains upto 7 % bergenin as an active constituent.

4. *Boenninghausenia albiflora* (Hook.) Meisner

Rutaceae, Flea Plant (Engl) and Yerma Shing (DZ).

A perennial nearly glabrous herb, 1m high, Leaves 2 - 3 pinnate, gland dotted, leaflets obovate or obcordate, 1- 2 cm, entire, Flowers bisexual white, 0.7 cm long, in terminal leafy panicle.

Fl. & Fr. : July to Nov.

Altitude : 1350 - 3050 m.

Distribution : Thimphu, Paro, Punakha, Tongsa, Shemgang, Trashigang, Phuntsholing, Upper Mo chu

Uses : The plant is believed to cure headaches if is kept under the pillow during sleeping hours. According to Manandhar (1989) the leaf juice has strong disagreeable pungent smell. It is used to remove worms from wounds. The leaf paste mixed with water is used for washing the face of an ill person, in the belief that it provides

relief from fever. It contains 0.2 - 0.4 % essential oil. The dried leaves are crushed into a flea powder as they emit a strong foetid smell.

The entire aerial parts of *Boenninqhausenia albiflora* are used to kill flee, lice and other insects (Gupta, 1981). Dam and Hajra (1981) have reported that a paste made from the powdered root and water is applied to cure old wounds. According to Rizvi *et al.*, (1979) the coumarins from *B. albiflora* like bergapten and Chalepin acetate have shown a good degree of spasmodic activity.

The plant is suitable for cultivation on commercial scale.

5. *Docynia indica* (Wall.) Decaisne

Rosaceae, Thung churpu (Sh), Tong shing (Dz) and Mehel (Nep).

Small deciduous tree 4 -10 m. branches sometimes spiny. Leaves ovate 9 - 15×3.5 - 6 cm, acuminate, margin subentire, glabrous above, tomentose beneath. Flowers 1 - 3 at branch ends, unfoldings with young leaves. Fruit pome, greenish yellow, 2.5 - 4 × 2.5 cm 15 - seeded.

Fl. & Fr. : March - May.

Altitude : 1300 - 2400 m.

Distribution : Punakha, Tongsa, Mongar and Trashigang districts.

Uses : The fruit has medicinal value. It is used by local people in dysentery.

6. *Drymaria cordata* subsp. *diandra* (Blume.) J. Duke

Caryophyllaceae, Avizalo (Nep).

Diffuse glabrous herb, 30 - 70 cm long, stem branched from the base. Leaves opposite, 1.6×1.6 to 2.6×2.6 cm, Ovate or orbicular, 3 - 5 (rarely 7) nerved, Stipules 3 - 5, bristly. Inflorescence axillary and terminal cyme. Flowers white 6 mm in diameter, Capsule ovoid, trigonous, Seeds one - many, globose, Finely tubercled.

Fl. & Fr. : August - November.

Altitude: 300 - 2000 m.

Distribution : Samchi (Dorakha) Gaylegphug, Tama and Trashigang (Kanglung).

Uses : The plant is an effective medicine of cough and cold. The local people smash the plant and mix with hot ash and wrap in a fine cloth.

The hot air (heat) coming out is inhaled in case of severe headache, cough and cold. An ethnobotanical study done by Manandhar (1989) and Gupta (1981) also emphasize the medicinal properties of this species. The plant juice is administered in conjunctivitis. Despite the plant being effective in cough, cold and headache. It is also used as a medicine for snakebite. (Biswas *et al.*, 1956).

In Meghalaya (India), this species is used as an effective medicine for snakebite by Garo tribes (Rao, 1981). The leaves are crushed and applied in case of snakebite. It is said the plant is even effective in case of deadly snakes. In addition to external application mentioned above the juice of the leaves may also be taken by the patients. According to Gupta (1981), the leaves together with *Ageratum conyzoides, Galinsoga parviflora, Bidens biternata* and ginger are made into a paste. It is applied as a remedy for snakebite. The leaves of *D. cordata, Scoparia dulcis* and salt together crushed and applied for eye sores (Kharkongar & Joseph, 1981).

7. *Potentilla fulgens* Wall. ex Hook.

Rosaceae, Bajradanti (Nep).

An erect herb upto 50 cm high. Leaves imparipinnate, leaflets 12 - 18 pairs, alternately large and small, ovate, silky, tomentose. Flowers yellow.

Fl. : July - Sept.

Altitude: 2250 - 3600 m.

Distribution : Thimphu, Punakha, Tongsa, Bumthang, Mongar and Trashigang districts.

Uses: The plant juice is taken to treat stomach problems, cough and cold.

Manandhar (1989) has reported that the root powder is effective against toothaches and is also taken as an anthelmintic. The leaves when masticated are supposed to be beneficial for pyorrhoea.

8. *Rhododendron arboreum* Sm.

Ericaceae, Ito Meto (Dz), Zhu-dang Meto (Sh) and Gurass (Nep).

A medium size tree upto 15 m high. Leaves stalked, 4.5 - 16×1.5 - 6 cm oblong, lanceolate, narrowed at both ends, silvery beneath, crowded towards the end of the branches. Flowers red, crowded in large rounded corymbs. Fruits cylindrical capsule, longitudinally ribbed, about 2.5 cm long.

Fl. & Fr. : March - May

Altitude: 1200 - 3000 m.

Distribution : Chukha, Gaylegphug, Thimphu, Punakha, Tongsa, Bumthang, Mongar, Sakden and upper Mo Chu.

Uses: The petals of *Rhododendron arboreum* Sm. are given in dysentery by local people. Dried petal is also used in the treatment of throat and stomach disorders. While quoting Yonzone *et al.* (1981), Mukherji and Rai (1986) have emphasized the medicinal importance of different parts of this plant. Leaves when applied to forehead relieve headache. A jelly made from the petals is used in blood dysentery and throat pain. Dry flower powder is also used to expel splinters from throat. Manandhar (1989) reported that the plant juice is taken to treat dysentery

and diarrhoea and paste in case of headache. Flowers have been reported to have antidysentric property.

9. *Rubus ellipticus* Sm.

Rosaceae, Tshema Tshelu (Dz), Gongsey (Sh) and Aiselu (Nep).

A straggling shrub upto 5 m high drooping on the roadsides from hills, with rusty brown bristly hairs. Leaves 3 - foliate, 1.5 - 9×1 - 7 cm, terminal leaflet being the largest, Ovate - elliptic, serrate, apex rounded, hoary - pubescent beneath. Flowers white in dense axillary and terminal panicles.

Fl. & Fr. : Dec. - May

Altitude : 1200 - 1900 m.

Distribution : Samchi, Phuntsholing, Chukha, Gaylephug and Trashigang districts.

Uses: The plant is used as astringent. The economically useful part is the root and fruits. Root paste is applied to treat wounds, while a decoction of the root in water is used as febrifuge. The fruit juice is given to cure fever and is also considered useful in cases of cough (Manandhar, 1989). The root and young shoots are considered good for colic pain.

The plant has been reported to possess antifertility activity (Sharma *et al.*, 1981). An ethanolic extract of aerial parts of *Rubus ellipticus* (50 mg/kg daily for the first seven days of pregnancy) elicited 100% inhibition of pregnancy in rats. The root extract at 250 mg/kg dose also elicited a significant anti- implantation activity. Therefore the plant can be used as contraceptive or may be useful as an effective ingredient of any antifertility preparations.

10. *Schima wallichii* (DC) Korth.

Theaceae, Puyam (Dz), Chilaune (Nep), Aule Chilaune (Nep) and Zala Shing (Sh).

A large evergreen tree, height upto 25 m. Leaves stalked, 5 - 18×2 - 8 cm; Oblong - lanceolate, entire. Flowers yellowish white; attractive, fragrant. Fruits woody, loculicidal, capsule with persistent calyx. Seeds winged.

Fls: May - July.

Altitude : 300 - 2000 m

Distribution : Samchi to Gaylephug, Punakha and Trashigang.

Uses: The plant is highly medicinal. It is reported that the powder of the bark is made into paste and applied on the wounds of the cattle. Manandhar (1989) has reported that the bark of the plant is believed to be effective in treating gonorrhoea. The bark juice is applied to treat cuts and also taken in cases of gastric troubles. Kirtikar *et al.* (1935) have reported that this juice is anthelmintic and rubefacient. The powdered fruits are applied to treat scorpion stings.

A decoction of young leaves and roots is prescribed for fever (Storrs *et al.*, 1984). Chandel and Rastogi (1980 b) have isolated schiwallin which shows antifungal activity.

11. *Thalictrum foliosum* DC.

Ranunculaceae, Vansali, Mamira (Nep).

Robust bushy herb 1-2 (-4) m high. Leaves pinnately decompound,leaflets broadly ovate, 1.5 - 4×1 - 3 cm; acute or obtuse, toothed or lobed. Flowers white or yellowish or dingy purple in panicles.

Fl. & Fr. : June - Aug.

Altitude : 1370 - 2130 m.

Distribution : Deothang, Punakha, Tongsa, Mongar and Trashigang.

Uses: The roots are reported to be effective in toothache, piles and earache. A decoction of the root is a ready remedy for opthalmia and is also used as a febrifuge. (Manandhar, 1989). In India the root is used as a tonic; it contains berberine in a solution form (Bamber, 1916).

12. *Toricellia tiliaefolia* DC.

Cornaceae, Dhengboor Laga (Sh) and Lekh Bhogte (Nep).

A small deciduous tree with stout branches; leaves broadly, cordate, 10 - 15 cm wide, petiole upto 10 cm long, sheathing at base, Flowers unisexual, small, white, in pendulous panicles, male peduncle dense flowered about 30 cm long, females lax flowered, shorter. Fruit small, purple, obliquely ovoid, 1 - seeded.

Fl. & Fr. : April

Altitude : 1000 - 2200 m.

Distribution: Chukha, Punakha, Tongsa, Mongar and Trashigang district.

About the rarity of this plant Sahni (1981) writes that "a tree from Kumaon and Nepal whose rarity can be judged by the fact that there is no specimen of it in the herbarium of the north circle of BSI at Dehradun. The F R I herbarium Dehradun has only five specimens. Presently collected from Kameng District". However, in Bhutan this species is commonly found.

Uses: The fruit of this plant is used by local people to cure the stomach swelling. When stomach/belly portion swells and becomes hard, especially in case of ladies, fruits are crushed in water, made into paste and applied. It gives quick relief.

13. *Valeriana jatamansi* Jones

Valerianaceae, Chailpasu, jatamansi (Nep).

A perennial herb, 9 - 45 cm high, with thick rootstock. Radical leaves crowded near the base persistent, long petiolate, ovate - cordate, toothed or sinuate. Cauline leaves few, much smaller. Flowers in terminal corymbs, white. Fruit small, 1 - seeded, crowned with long plumose, calycinal bristles.

Fl. & Fr. : Feb. - May

Altitude : 1000 - 3500 m.

Distribution : Norfong, Wamrong, Kanglung and Thimphu.

Uses : Valerians are represented by only two species in Bhutan, namely *V. jatamansi* jones and *V. hardwickii* Wall, and both these species are medicinally important. However in India some other species viz. *V. officinalis* Linn. and *V. arnottiana* also occur which have significant medicinal value. The roots of *V. jatamansi* are locally used to cure hysteria, epileptic fits, headache and eye troubles (Subramanyam, 1973). According to Rao (1981) the plant juice is applied to counteract the poison of insects and scorpions. The pounded levels or roots of *V. hardwickii* are used on boils. The root is stimulant carminative and is used to treat rheumatism and rheumatic fever (Manandhar, 1989).

Jain (1983) has reported that *V. officinalis* Linn. has depressant action on central nervous system and is used in the treatment of hysterical fits, other nervous disorders and flatulence. The fresh juice is used as a narcotic in insomnia and in certain cardiac preparations.

Mary *et al.*, (1981 b) have conducted pharmacognostical, botanical and phytochemical studies on *V. arnottiana*, a south Indian Species. They compared *V. arnottiana*, with *V. jatamansi* and have provided with distinguishing features to be used in the field and in crude drug form. The volatile oil present in *V. arnottiana* is 2.6% as compared to 2.2% in *V. jatamansi*. They have also suggested that *V. arnottiana* may serve as a useful alternate source for "Indian Valerian" *V. jatamansi*. On the basis of phytochemical analysis of root and rhizome of *Nymphoides macrosperma* and *V. jatamansi* Mary *et al.* (1981 a) have suggested that *N. macrosperma* can very well replace Indian Valerian i.e. *V. jatamansi*. They have also pointed out the differentiation features of both these drugs in crude form.

Indian Valerian obtained from *V. jatamansi* has been indiscriminately exploited from its natural habitat and is facing endangerment in India (Mehrotra *et al.*, 1986). Valerians are so important drug and the climatic conditions of Bhutan are very congenial for their luxuriant growth, that they can be easily cultivated on commercial scale and may proven to be a good source of foreign exchange.

14. *Viscum nepalense* Sprengel

Loranthaceae, Ngenshi Thup (Dz), Ngashing Jormu (Sh) and Harchur (Nep).

A semiparasite, shoots upto 1 m, terete only near base, di or trichotomously branched above, constricted at nodes, internodes flattened. Inflorescence borne at

nodes, female flowers 1 - 3, subtended by cup-shaped bracts which is followed by several male flowers. Fruit globose, yellowish.

Fl. & Fr. : May - Aug.

Altitude : 1400 - 2400 m.

Distribution : Deothang, Punakha and Trashigang districts.

Uses: The plant is said to be useful in the treatment of rheumatism and fracture. The stems are crushed with water to make a paste and then applied on the fractured part of bone. Decoction of the plant is also taken internally to cure bone fractures. The plant is reputed to be very effective.

15. *Woodfordia fruticosa* (L.) Kurz

Lythraceae, Zange Shing (Dz) and Dhaneyaro (Nep).

A shrub with spreading and drooping branches, Height 2 - 3 m. Leaves sessile, 5 - 12×1.5 - 4 cm. Opposite rarely whorled in three, Oblong - lanceolate, entire, usually acuminate. Flowers in axillary cymes, bright brick-red.

Fl. & Fr : March - June.

Altitude : 610 - 2000 m.

Distribution : Gaylegphug, Punakha, Tongsa and Trashigang districts.

Uses: The fruits are boiled in water and the decoction is given for dysentery by local people. The flowers decoction is useful in constipation.

According to Manandhar (1989) the flower boiled in water is given to control profuse menstruation (Menorrhagia) and is also given to treat fever. The flowers are also known as astringent. (Bamber, 1916).

Pal (1981) has reported that in Orissa (India) the Lodhas apply the decoction of the leaves on sores and on the ulcers of cattle. Possibly due to high tannin content in the leaves (12 - 20%) the ulcers are cured. Jain and De (1966) have reported the crushed roots are given to cattle for rheumatism. Flowers are considered a tonic. A paste of the flowers is given in cough; it cures in two or three days. A decoction of five or six flowers per dose is given with honey for 3 to 4 days in nausea and aversion to food during pregnancy. It is also used in small pox (Jain, 1981).

Shome *et al.*, (1981) have carried out pharmacognosy of the flowers of *Woodfordia fruticosa* to laydown standards for the genuine drug. The study includes the detailed anatomy of the different parts of the flowers, pollen ornamentation, seed with unusual mucilage hairs and embryo characters. The plant is so useful that there is a need for further researches and can be cultivated on commercial scale after being thoroughly investigated.

Conclusion

The present study reveals that a considerable amount of knowledge on the traditional uses of plants is available with people in the villages. Although efforts were made to collect maximum knowledge about the medicinal uses of plants but due to scattered population and difficult terrain, it is felt that much information about the medicinal uses of plants could not be unravelled. The flora of Bhutan is very rich but depletion of natural resources including vegetational wealth all over the World is a regular feature (Ramakrishnan, 1984, 85; Mayers, 1980; Oldfield, 1981, 84). Bhutan in no way is an exception to this global phenomenon. During the course of study it was also surprisingly observed that in many parts of the country the medicinal plants are used as food and fodder only and therefore these are not properly utilized which is probably due to lack of information and knowledge about them. The communities from the remote villages always prefer to restrict their dependence on the herbal medicine only. But due to progressive modernization all over the World the usage of ethnomedicinal value has been declining and therefore a great need for conserving wild genetic resources (Alcorn, 1981 a, 81 b, & 84 and Gangawar and Ramakrishnan, 1990) and the knowledge of herbal treasure, has been emphasized.

As plants are used in various ways in different parts of the country, it is suggested that a comparative study of uses of plants by various communities in Bhutan would certainly provide more ethnobotanical knowledge. The information regarding the medicinal uses of plants must be confirmed by pharmacological, pharmacognostical and clinical researches. It is quite possible that some plants may prove to be extraordinarily useful due to its chemical constituents. Therefore some plants might become a source of revenue for the country. While there is no report of proper cultivation of medicinal plants in Bhutan, cultivation of some medicinal plants viz. *Valeriana* sp., *Boenninghausenia albiflora* on commercial scale has been recommended.

Acknowledgement

The author records his deep sense of gratitude to Dasho Zangley Dukpa, the Principal, for encouragement and for providing necessary facilities. Grateful thanks are also to Dr.G.N.Dixit, my colleague in the department for his valuable suggestions.

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Caesalpinia crista - A Potential Ethnomedicinal Plant

V.Gopal & Malati, G. Chauhan

Deptt. of Pharmacognosy
L.M.College of Pharmacy
Ahmedabad

Introduction

CAESALPINIA crista L. (Caesalpiniaceae), is a medicinal plant growing wildly throughout India and tropical countries of the World[1]. The plant was much confused with *Caesalpinia bonducella* (Syn. *C. bonduc*) and was described under the same [2,3,4,5,6,7,8]. Besides this species like *C. nuga* [1,3,4,9,10] and *C. jayoba* are also sometimes wrongly designated as synomyms for *C. crista*. In fact, *C. jayoba* is an adulterant of *C. crista*[7].

Each and every part of the plant is claimed to possess some therapeutic property but seed kernel is the most widely used part all-over the world in various systems of medicine. Seeds are extremely bitter, commercially available in plenty at a very low cost and are widely used for a variety of diseases, especially in cases of all types of fever including malaria.

Nomenclature

The name of the species "Bonducella" is derived from the Arabic word "Bonduc" meaning a "little ball" which indicated the globular shape of the seed. In English it is commonly named as "Fever-nut". "Grey Wicket bean", "Indian nut", etc. which indicated the nature and properties of the seed. Its Sanskrit name "Kumberakshi" meaning "eye-ball" is referred to the shape of the seed while the Marathi name

"Sargargota" meaning polished round stone of the sea, referred to the hard, glossy, globular seed of *C. crista*.

Distribution and Habitat

Fairly common throughout India in tidal and beach forest and along tidal river banks, as well as banks of streams and rivers in island forests of Orissa and Western sea-coast from Konkan southwards, and also in Sunderbans of Bengal, Bhopal and Himachal Pradesh. It is propagated from seed which exhibit dormancy but can be overcome by acid scarification, light, temperature or sulphuric acid treatment[1].

General Features

C. crista L. is a large, woody, prickly shrub attaining a height up to 15 m or more. The plant looks attractive because of its yellow flowers and glossy bipinnate leaves consisting of 6-8 pairs of pinnae (Fig. 1). Each pinna is composed of 6-9 pairs of leaflets which are membranous, elliptic, oblong, obtuse and strongly mucronate. Flowers are in dense long peduncled terminal and auxillary racemes. The plant bears flowers from July to September and fruits from September to December, seed gets ripened from February to April.

Fig.1 : Caesalpina Crista

(a) A Twig

(b) Fruit

Ethnomedicinal Properties

The plant possesses number of therapeutic properties like antipyretic, antiinflammatory, anthelmintic, etc. The therapeutic applications of individual parts of the plant are discussed below 1,12,13,14,15,16 and 17.

Seed

The kernel of the seed is very useful and valuable in all ordinary cases of simple, continued and intermittent fevers. It is commonly used by natives as a substitute of Quinine. The kernel powder mixed with equal parts of black pepper is taken thrice a day in a dose of 15-30 grains by adults and 3-4 grains by children. It was made official in the Indian Pharmaceutical Codex 1953[18] the dose of the powder being 15-18 grains. It is said to produce lots of perspiration, leading to the reduction of fever. The antiperiodic property of kernel is not as effective as Quinine but it certainly stands next to Quinine. "Bonducin" the bitter principle of the drug clinically, is claimed to possess good antimalarial properties[16].

In smaller doses (5 grains) it is a good tonic in debility after fever and other diseases. For these purposes it is usually prescribed either with Kalijiri (*Vernonia anthelmintica*) or with warm butter milk and asafoetida. As a bitter tonic it may be used as a substitute for Gentian. In Malaya, seeds are used as an astringent, tonic, in cases of bowel complaints and said to facilitate child birth. It is found to be useful in convalesence and spasms after fever and may be used as a substitute for Valerian. In hysteria, its powder is prescribed with jaggery or with cadamom, jatamansi and honey.

The antiinflammatory property of kernel is also well-established. An ointment prepared from seed kernel and Castor oil or the extract of the powder in the form of a paste, when applied externally in cases of hydrocele and glandular swellings gave excellent results. Oral administration of the drug (30 grains of powder roasted in castor oil) also gave good results in hydrocele. In Madras state, use of the powder with castor oil orally to remove swellings from different parts of the body like stomach, legs, hands, heart, etc. is well- known indicating good diuretic activity of the kernel. In some parts of Kerala, powder of the baked kernels with honey is used in the treatment of hernia and swelling[19]. Seeds are used in cases of rheumatism.

Kernel powder with sugar and goat milk gives good results in liver disorders[20]. Decoction of the roasted kernel was used in asthma. Seed and long pepper powders taken with honey gives good expectorant effect. Children unable to digest mother's milk were given the extract of the kernel or its powder along with ginger, salt and honey to get good stomachic effect. Paste prepared from the kernel gives relief from boils and other such swellings. Seeds are useful for keeping off infectious diseases like leprosy. Burnt seeds with alum and burnt arecanut is a good dentrifice useful in spongy gums, gum boils, etc. A cake made of 30 grains of powdered kernel, fried in ghee taken twice a day is a valuable remedy in cases of acute orchitis, ovaritis

and scrofula. The seed is claimed to be syptic, purgative, anthelmintic[21] and is used as an antidote against opium, aconite, arsenic and copper poisonings. It is recommended as a single drug for the treatment of amoebiasis[22]. In West Indies, the roasted seeds are used as antidiabetics[1]. The ethnomedical survey by the authors around certain regions of Gujarat revealed the use of the decoction of the kernels in cases of diabetes.

Fixed Oil

Fixed oil expressed from the seed kernel is emollient and is used as an embrocation to remove freckles from the face, as a cosmetic and also for stopping discharges from the ear. The oil is said to soften the skin and remove pimples. It is useful in cases of rheumatism, convulsions, paralysis and similar complaints. It is prescribed in cases of leucoderma, boils, and other skin infections.

Leaves

Leaves after roasting with castor oil are applied externally to inflammatory swellings especially to inflammed piles, hydrocele and orchitis with benefit. Tender leaves boiled with castor oil or ghee, if thickly applied on painful and swollen testicles are found to be very efficacious. It also gives relief in cases of rheumatism. Leaves ground with onion, if applied on plague boils gives good relief. In disorders of the liver, the tender leaves are considered very efficacious. In China, they are reckoned as a deobstruent and emmenagogue. Leaf extract with rock salt or leaf paste with salt is given to patients suffering from liver disorders. Finely powdered leaves are prescribed as a uterine tonic after child birth. The warm decoction of the leaves is used as a gargle for sore throat. In Ceylon, they are applied for toothache and also given to children for expelling worms. In Malaya, the tender leaves are used in intermittent fevers and for expelling intestinal worms. The juice of the leaves is anthelmintic, deodorant and good in elephantiasis and small pox. The leaf extract with asafoetida is prescribed in cases of indigestion.

Root

Bark of the root possesses number of properties like emmenagogue, febrifuge, anthelmintic, etc It is said to possess diuretic and purgative properties also and is useful in removing gravel and stone from the bladder. In Jamaica it is used as rubifacient and as a local application for sores. In Madagascar, the roots are considered febrifuge and anthelmintic and are much used as an astringent in leucorrhea and blenorrhagia. Powdered roots with honey are taken as a cure for leucorrhea[23]. In Guinea, the decoction of the root is prescribed in fever. Bark is used in tumours and for removing placenta. The bark powder with honey is taken in cases of hernia[24]. In Himachal Pradesh, the roots are used in intermittent fever and diabetes.

Fruit

The fruit is astringent to the bowels, aphrodisiac, anthelmintic, cures urinary discharges, leucorrhea, piles and wounds. The oil of the fruit is good for indolent ulcers. Roasted fruits are used in eye diseases, hyperacidity and as fish poison. In Hawaiin islands, the pulp of the pod is used for purifying the blood, in congestion and as a laxative. The pulp is reported to possess piscicidal properties also[25]. In Philippines, fresh fruit powdered with garlic and mixed with lukewarm water is rubbed on the body to mitigate fever[26].

Flower

It is expectorant and is used in cases of "kapha" and "vata". The ash is used in ascites.

Stem

The stem is used in eye diseases and as fish poison. In Sri Lanka, the plant is used for the treatment of skeletal fractures.

Macroscopy of the Seed[18,27,28]

Seeds are oval to globular, slightly flattened and about 2-2.2 cm in length and 1.2-1.5 cm in breadth. One edge of the seed is broadly rounded and gets gradually narrowed at the other edge. Seed coat is hard, glossy, greenish to ash grey in colour and is traversed by circular and vertical faint markings of the cracks, forming uniform rectangular to squarish reticulations all over the surface. A raised hilum with remains of the stalk lies in the centre of the dark spot, at the narrow edge of the seed. Adjacent to the hilum, lies a faint coloured circular to oval elevated micropyle In dry seed, kernel gets detached from the testa. Testa is about 1-1.25 mm in thickness and is composed of three distinct layers, the outermost - thin and brittle, the middle one - broad, fibrous and dark-brown and the innermost - white and papery.

The seed is exalbuminous. The kernel is hard, pale yellowish- white, circular to oval, flattened and about 1.25-1.75. cm in diameter. The surface is furrowed and ridged..A scar of the micropyle lies at one end of the kernel, from where arises a prominent ridge demarking the two cotyledons of the embryo. Plumule - radicle axis is thick, straight and cylindrical. Taste is very bitter and odour is unpleasant and nauseating.

Microscopy of the Seed[27]

The outline of the section is trigonous to almost rounded. The outer testa is composed of a layer of palisade cells, a layer of bearer cells underneath this and the

innermost many layered thick walled parenchymatous tissue. The middle testa is composed of many layered thick walled parenchymatous tissue, filled with brown resinous content and traversed by vascular bundles. Inner testa is composed of 6-7 rows of small, thin walled, compact, parenchymatous cells. Parenchymatous cells of the cotyledons are thin walled and contain reserve food material like starch, fixed oil and aleurone grains.

Columnar palisade cells, bone shaped, thick walled parenchymatous cells with brown content and cells filled with starch grains are some of the major diagnostic microscopical characters of the powder.

Phytochemical Investigation[29]

Even though each and every part of the plant is claimed to possess some therapeutic properties, seed kernel alone has been systematically studied so far.

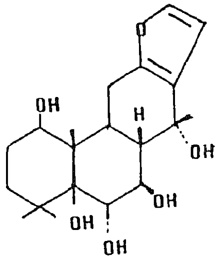
Glycosides

Bonducin (Bonducellin)[30], was the first non-alkaloidal bitter principle isolated[31] from the seed of *C. crista*. It was detected as a glycoside[32] and was said to be a sulphur containing compound. But later on[33], the compound (C_{20}, H_{28}, O_8 - m.p. 119.20°C) was found to be devoid of sulphur. The structural formula of Bonducin (a homoisoflavone) has been well-established recently[35] (Fig. 2). Seed was reported to contain saponin[33,34] but later on was found to be devoid of this[36]. Twigs of the plant contain steroidal saponins[37]. Number of enzymes like protease, urease, amylase, peroxidase, catalase and oxidase have been reported in the seed[38].

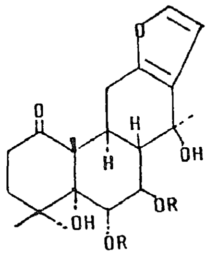
Alkaloids

Controversial reports existed regarding the presence of alkaloids in *C. crista*. Earlier workers detected an alkaloid "Natin" in the plant but could not confirm its presence[34,39]. Later reports indicated the presence of alkaloid in the seed[40] and twigs[37] and its absence in stem and leaf.

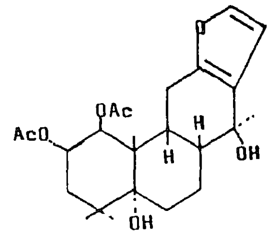
Terpenoids (1,5,6,7,14-Voucapanepentol derivatives) : (Fig. 2) Caesalpin ($C_{24}H_{32}O_8$) (1-ketone 6,7-diacetylcassane) M.W. -448.512, β-caesalpin ($C_{20}H_{28}O_6$) (1-ketone 5,6,7,14-tetrahydroxy voucapanone) M.W.-364.438 and α-caesalpin ($C_{34}H_{56}O_7$) (o-tetradecanoyl voucapane diterpenoid) M.W. -576.812 were the first three bitter cassane/voucapane diterpenoids isolated from the seeds of *C. crista*[42-44]. Determination of the functional groups[41], other chemical aspects[45], structure elucidation[46,47,48,49], etc. were exhaustively studied by number of workers. δ-caesalpin ($C_{20}H_{30}O_6$) (1α, 5α, 6α, 7β, 14β-cassane) M.W. - 366.453 is a hydrolysed product of γ-caesalpin and a reduced product of α-caesalpin[48] and β–caesalpin. α-caesalpin on hydrolysis yields acetic acid, myristic acid and a crystalline bitter compound ($C_{20}H_{30}O_6$). The structural relationship of α, β and γ caesalpins with vinaticole, vouncapenic and cassaic acids have been established[50]. Three more Caesalpins—E



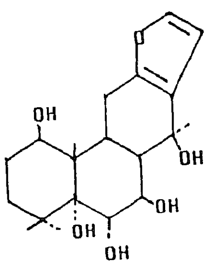
1,5,6,7,14
Vouacapanepentol



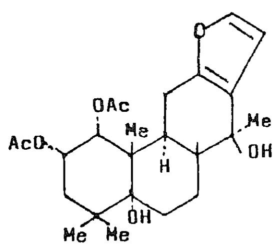
α-Caesalpin
R = AC
β-Caesalpin
R = H



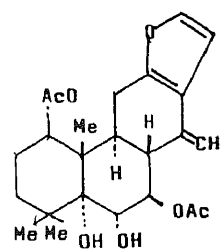
γ-Caesalpin



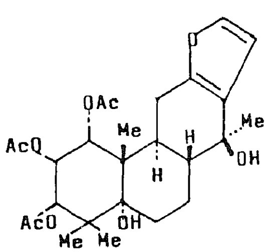
δ-Caesalpin



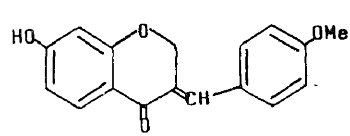
ε-Caesalpin



φ-Caesalpin



F -Caesalpin



Bonducellin

Fig. 2

caesalpin, Y caesalpin and F caesalpin have also been isolated from the kernel of *C. crista*. Y caesalpin, a minor constituent, is closely related to δ-caesalpin[51] and F caesalpin is closely related to E caesalpin[52].

Reserved Food Materials of the Kernels

The kernels contain about 20-25% of fixed oil, 36% protein and 50% carbohydrate. Fixed oil of the kernel is thick, pale yellow, with disagreeable odour and bitter taste. The constants of the oil are : sp. gr. 30^{o} - 0.926, n^{28} - 1.4745, sap. val. -197.9, iodine val. - 111.0, acetyl val. -35.6, acid val. -8.5 and unsaponifiable matter -1.1%. Figures of the earlier reports of this are slightly different[32]. The oil contains fatty acids like palmitic (10%), stearic (6%), lignoceric (61%), oleic (21%), linolenic and a mixture of unsaturated acids of low molecular weight [1]. Two phytosterolins (one identical to puranol), two phytosterols (one was sitosterol) and heptacosane have been isolated from the oil[33,54].

The percentage of protein content in the seed kernel of *C. crista* varies from 7.4[36] to 18.4[54] to 25.3[55]. The composition of amino acids was studied by number of workers[54-56]. It contains:

aspartic acid - 9.5%, arginine-3.3%, L-alanine-2.5%, r-amino- butyric acid-3.7%, citrulline-3.6%, cystine-1.2%, glycine-6.9%, glutamic acid - 3.6%, histidine-5.1%, leucine-6.3%, isoleucine- 5.1%, lysine-7.9%, methionine-2.1%, phenyl alanine-1.4%, proline- 3.3%, serine-3.8%, threonine-3.6%, tyrosine-3.7%, tryptophan-0.8% and valine-1.2%.

The amino acid substrate specificity of glutamyl-t-RNA synthetase prepared from the seed was also studied[57]. The non-protein amino acids detected in the seed were r-ethylidene glutamic acid, r- methylene glutamic acid, r-ethyl glutamic acid and traces of r-OH-r-methyl glutamic acid and B-OH-r-methyl glutamic acid, accumulations of r-methyl glutamic acid being extremely large[58]. Seed protein of Caesalpinaceae was studied with chemotaxonomic view point by number of workers[59-62]. Starch (6.1%)[63], sucrose[33], pentosan (16.8%)[36] and water soluble mucilage (4.4%)[36] are some of the common carbohydrates reported in the seed. 4-o-methyl myoinositol hydrate was isolated from *C. crista* grown in China.

Leaves

An amorphous bitter principle (C_{24}, H_{32} O_8, m.p. 119. 120, yield 0.35%) and a waxy material have been isolated from the leaves[64]. The waxy material on saponification yields myristic acid and an alcohol. The leaves also contain glucose, pinitol (4.1%) and minerals like calcium (2%) and phosphorous (0.3%).

Pharmacological Investigations

The bitter principles are said to be responsible for the pharmacological activities of the drug but except β-caesalpin, none of the other principles have been found to be tested so far.

The earlier reports mentioned the non-glycosidal bitter principle to be pharmacologically inactive[65] and the bitter principles of the alcoholic extract of the kernel to be ineffective against bird malaria[66]. Later reports indicated[67], the pharmacological activity of the aqueous and alcoholic extracts of the seed, their bitter principles and four isolates (fractions A, B, C, D) on various tissues and systems. The alcoholic extract lowered the blood pressure in dogs whereas the aqueous extract had no effect. The alcoholic extract depressed the heart and diminished the amplitude of contraction, while the aqueous extract had the same but less marked effect. Both the extracts did not show any action on the blood vessels and uterine muscle of the rat, on the central nervous system of the cat, and on antipyretic and antidiuretic action on rabbit. The bitter principle and its fractions A, B, C and D did not show any actions on the blood pressure or on the heart of frog. Fraction A slightly contracted rectus abdominis muscle, and exhibited a more profound antispasmodic action on an already contracted rat ileum. Fraction B showed marked depression activity on the central nervous system of frog. Only fraction B showed significant diuretic activity. The total bitter principle exhibited antipyretic activity but fraction B exerted a more marked action. The aqueous and alcoholic extracts did not show anthelmintic activity but the total bitter principle had a marked action, and fraction D was found to be strongly anthelmintic.

Clinically even though the kernel has been claimed to be efficacious in cases of intermittent fevers[68] pharmacological investigation did not give positive results[69]. B caesalpin one of the bitter principles of the kernel has been now proved to be antimalarial in action at a dose of 1.6 mg/kg body weight both by p.o. and s.c. in a rodent test system injected with *Plasmodium berghei*[70].

Seed showed antiinflammatory activity in rats at a dose of 250 mg/kg body weight by granuloma pouch method[71]. It has also been proved to possess antidiarrhoeal activity[40].

Antibacterial, antifungal and similar other activities of seed and leaf were studied by number of workers. The aqueous and alcoholic extracts of the kernel showed good antibacterial activity against *M. pyrogenes, E. coli* and *S. paradysentericae* while the fraction (A, B) isolated from them showed activity against *E. typhosum, B. subtilis* and *S. paratyphi A* and *B*. The other fractions were inactive[67]. The aqueous extract of the leaf was studied for antibacterial activity against *S. lutea*, antifungal activity against *A. niger*, antimycobacterial activity against *M. phlei* and anti-yeast activity against *S. cerevisiae* and found it to be inactive against these microorganisms[68]. The aqueous extract of the leaf showed strong *Antinematodal* activity against *M. incognita*[72].

Oil extracted from the kernel exhibited good antibacterial activity against *B. subtilis, B. megatherium, S. aureus, S. typhi, S. dysentericae, P. vulgaris, C. albicans, R. glutinus, A. wantii* and *P. chrysogenum* organisms[73]. The activity was noted to be present in the oil obtained from long preserved batch of seeds. The oil obtained from the fresh seeds does not possess antimicrobial activity. The oxidised oil possessed the same degree of antimicrobial activity as that from the old batch of seeds. This suggested that during the preservation of the seeds, slow oxidation of the component unsaturated fatty acids occurred and these oxidised fatty acids showed the antimicrobial activity.

Clinically the seeds were tested in 10 patients suffering from intestinal amoebiasis at a dose of 2 g thrice a day for fifteen days and found to yield excellent antiamoebic activity[22].

Benzindenopyran derivatives obtained from the extracts of Caesalpina plant was found to be useful for the prevention of microangiopathy and for the stimulation of blood microcirculation[74].

The authors studied the aqueous and alcoholic extracts of the kernels of *C. crista* for its effect on the blood sugar level of rats in normal fasted model and glucose loaded model (OGTT) and found both the extracts to show significant hypoglycaemic activity. Standard Ayurvedic texts recommend the roasting of the seed prior to removal of its kernels[75,76]. It was also found that the Ayurvedic drug manufacturers follow the practice of roasting the seeds in order to make easy the task of removing the seed coat. Exposure to high temperatures during roasting may inactivate the pharmacologically active principles of *C. crista*. Hence the effect of high temperatures on the hypoglycaemic activity of the kernels was studied. Roasting of the seed was found to reduce the hypoglycaemic activity of the kernel but the decrease in activity was not statistically significant.

Comments

Caesalpina crista (Caesalpinaceae) is a widely growing common plant, available in plenty in many regions of India. Seeds are sold in the market at a very low cost and the kernels are very popular as a home remedy, for various diseases amongst the tribal people. People residing in Dang forest near Surat district of Gujarat appear to be aware of the hypoglycaemic activity of the kernel since long, because we have seen them prescribing the aqueous extract of the kernel to many people suffering from diabetes. Surprisingly no ancient text mentions about the hypoglycaemic activity of the kernel but antidiabetic formulations manufactured in Gujarat do contain seed kernel as one of the ingredient. Few local Ayurvedic physicians are found to be prescribing kernel powder alone to diabetic patients to lower down the sugar level. These observations led us to investigate the hypoglycaemic properties of the kernel clinically and pharmacologically. Clinical studies would give a better picture about this and the side effects of the drug if any. Authors have undertaken

the project of investigating the hypoglycaemic activity of the primary metabolites especially protein fraction of the kernel.

Some authorities claim root bark of *C. crista* to be equally efficacious as kernel but as yet these claims have not been investigated. Individual bitter principles of the kernel also need to be investigated. Literature survey indicated that *C. bonducella* is a synonym of *C. crista* and the plant was investigated as one species. However, Wealth of India (1992), incorporated *C. crista* and *C. bonducella* as two separate plants. Both the plants are known under the name of "fever nut". Whatever may be the controversy, the authors feel that the plant needs a more thorough investigation.

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In Vitro Conservation of Diversity in Medicinal Plants of North-West Himalaya

K.P.S.Chandel & Neelam Sharma

National Plant Tissue Culture Repository
NBPGR, New Delhi - 110 012 (India)

MEDICINAL and aromatic plants have been the subject of man's curiosity and usages for centuries, ever since man began settled life some 8000-10000 years ago. Indian gene centre has been the seat of ancient civilization and several plant species were domesticated in the dim antiquity of past. Indigenous medicine system "Ayurveda" goes back along the Chinese and Unani system of medicine and even today it is highly relevant and effective in the cure of serious ailments and health problems. The ancient man totally depended on medicine produced from various plant parts and plentiful description and usages of plant species can be found in ancient literature. It has been a great heritage passed on from generation to generation. The countries in the developed world have suddenly realized the importance of herbal drugs and several pharmaceutical formulations for the manufacture of drugs for serious ailments such as heart problems, cancer, leukaemia, diabetis and AIDS are being sought from plant products. It is estimated that some 300 plants are largely used in the preparation of medicines in India and more than 15,000 formulations are in use for various health problems and health cures. It is considered that there are some 40,000 registered manufacturers of Ayurvedic medicines in India engaged in the production of herbal medicines. According to recent estimates, native system of medicine (Ayurveda, Unani and Sidda) make regular use of over 1100 plants collected from wild state occurring in natural/disturbed habitats (Gupta, 1988). In recent years due to growing demand of plant based pharmaceutical products, the remarkable interest in modern pharmacological drugs has become evident. At

present, nearly 30 percent of the modern pharmaceutical drugs are derived directly or indirectly from plants (Balandrin *et al.*, 1985). Also in homeopathic/ayurvedic medicines, plants and their parts/extracts dominate the scene.

Richness of Biodiversity

Indian gene centre holds rich natural diversity of plant species occurring both in tropical/subtropical and temperate/alpine regions. The variability occurring in north western Himalayas is also very distinct as compared to the diversity occurring in the peninsular India comprising both Eastern and Western Ghats. Unfortunately, the plant populations of such useful plants have not been made to domesticate or improve through breeding and selection despite being in high demand at national/international markets. Thus, until now, only wild populations have constituted the major genetic resources (bulk material) for use in the indigenous system of medicine and pharmaceutical industry.

Causes of Genetic Erosion and Concerns for Conservation

Continuous, often indiscriminate and ruthless collection of plant populations in bulk quantity from diverse ecosystems coupled with destruction of natural habitats are resulting in irreplaceable loss of plant species According to Nayar and Sastry (1987), about 1500 plant species are threatened with extinction in India and of these 100 have been declared as rare plant species. Paradoxically, medicinal plant species account for one third of the species in the 'Red Data Book of India'. According to one estimate, 14 species are endangered or characterised as rare and seem to be under immediate danger. Besides, 35 medicinal plant species are vulnerable (Gupta, 1988)

The collection of these plant species need to be undertaken and encouraged only on a scientific basis so as not to damage the natural ecosystem and the accompanying species. The forest safety of the resources is linked with the sustainability of the habitats in which they occur and the quantum, rate and frequency of exploitation. Thus, the best approach may be to protect and preserve natural ecosystems by establishing "Nature Reserve" (Genetic Reserve) embodying the concept of *in situ* conservation. This strategy may require complementation through *ex situ* conservation methods, such as gene banks, *In vitro* repositories, cryopreservation banks, pollen banks and adequate linked field gene banks and herbal gardens. This may be necessary for those rare and endangered taxa, whose number of individuals and populations have declined to critical low levels.

Conservation Strategies

The conservation of orthodox seed species (desiccation tolerant) at low temperature in gene bank is very effective and ensures safe conservation for fairly long time (5-50 years) depending upon the species. However, very little is known about the mode

of regeneration (propagation) and storage potential of the propagules of the wild species. Development of tissue culture (*in vitro*) technology offers a great potential for rapid multiplication and short to medium term conservation of plant species of medicinal importance. Tissue culture techniques also provide an ideal means for establishment of large scale herbal gardens or the reintroduction of the plants in its natural habitats. Such views have also been emphasised by several other workers (Bhojwani *et al*, 1989; Lal *et al.*, 1988). Besides, the reduced cost and space requirement, ease in transportation of *in vitro* cultures or plantlets to distant places, without much quarantine restriction is another advantage. The *in vitro* cultures could be preserved overtime and multiplied as and when required.

Use of *In vitro* Technology

Until recently tissue culture studies on medicinal and aromatic plants were largely restricted for the production of desired secondary metabolites through cell suspension, or callus system During the past few years, the interest in mass propagation of medicinal plants threatened with extinction has distinctly increased (Tables 1&2). Further growing awareness and concern today is being expressed at various strata of our society. It is true that conventionally, these plants take a long time for multiplication, have a low rate of fruit/seed set, poor seed germination/viability and often roots/rhizomes accumulate active ingradient (principle) only when they have attained specific growth/developmental stage over time. Contrary to this, *in vitro* rate of multiplication in reported system varied from 3.5 fold/3 weeks in *Saussurea lappa* (Arora & Bhojwani 1989; Bhojwani *et al.*, 1989) to as high as 150 shoots per 4 months in *Coleus forskohlii* (Sen and Sharma, 1991) as shown in Table 1 The mode of propagation in most of the systems is through axillary branching except in *Picrorhiza kurroa* (adventitious shoots without callus formation (Lal *et al*, 1988) In *Podophyllum hexandrum* direct regeneration could not be achieved, although somatic embryogenesis was induced successfully (Arumugam, 1989; Arumugam and Bhojwani, 1990). In *Valeriana wallichi*, mode of regeneration was organogenesis through callus (Mathur & Ahuja, 1991; Viola & Fritz, 1991).

Rapid Multiplications of Endangered Himalayan Medicinal Plant species

Nardostachys jatamansi DC, a plant with high vegetative propagation potential occurring in the natural habitats (between 3300 to 5500 m altitude) in the Central and Eastern Himalayas has become endangered and scarce due to indiscriminate uprooting. The species has low seed viability, thus alternative *ex situ* conservation method such as tissue culture technique were integrated for the micropropagation of this valuable plant species of medicinal importance. Callus cultures of *Nardostachys jatamansi* DC maintained on Murashige and Skoog's medium containing 3 0 mgl^{-1} of napthalene acetic acid and 0.25 mgl^{-1} of Kinetin when shifted to

Table 1 : Status of *in vitro* conservation of threatened and endangered medicinal plants of NW Himalaya

Plant species	Mode of *in vitro* multiplication	Storage period and conditions	References
Aconitum heterophyllum	Somatic embryos	-	Giri *et al.*, 1992
Coleus forskohlii	Axillary shoots	-	Sharma *et al.*, 1991 Sen & Sharma, 1991
Gentiana kurroo	Axillary shoots	-	Sharma *et al.*, 1993
Nardostachys jatmansii	Callus mediated	-	Mathur, 1992
Picrorhiza kurroa	Adventitious shoots Axillary shoots	24 months at 4-10°C 10-14 months at 5°C	Lal *et al.*, 1988 Upadhyaya *et al.*, 1989
Podophyllum hexandrum	Adventitious shoots Somatic embryo	7 months at 5°C	Bhojwani *et al.*, 1989 Arumugam, 1989 Arumugam & Bhojwani, 1991
Rauvolfia serpentina	Axillary shoots	15 months at 15°C	Sharma & Chandel, 1992a
Rheum emodi	Axillary and adventitious shoots	-	Lal & Ahuja, 1989
Saussurea lappa	Axillary and adventitious shoots	12 months at 5°C	Arora & Bhojwani, 1989 Bhojwani *et al.*, 1989 Bhojwani *et al.*, 1989, 1992 b
Valeriana wallichii	Axillary shoots Callus mediated		Mathur *et al.*, 1988 Mathur & Ahuja, 1991

medium containing 0.25-1.0 mgl^{-1} of indole-3-acetic acid or indole-3-butyric acid showed profuse rhizogenesis (Mathur, 1992). The callus regenerated roots when transferred to medium containing 2.0-6.0 mgl^{-1} of kinetin produced *de-novo* shoot bud cells which originated from the inner stelar region. In addition, root-shoot development was also observed.

Valeriana wallichi D C is yet another important Himalayan plant species occurring between 1500-3000 m elevation. It contains sedative and tranquilizing principles (Valepotriates). The rapid multiplication through shoot tip and axillary bud explants was also reported in *V. wallichi* (Mathur *et al.*, 1988). Propagation of *V. wallichi* was reported using encapsulated apical and axial shoot buds (Mathur *et al.*, 1989) and plantlet development was observed both *in vitro* (98%) and *in vivo* (60%) in pots with vermiculite under controlled glass house conditions. In continuation of above work, petiole explants of *Valeriana wallichi* DC were used for inducing callus. Optimum callus formation was noted on Murashige and Skoog's medium supplemented with 3.0 mg/l NAA and 0.25 mg/l Kn. Shoot regeneration occurred on transferring the callus to medium containing 1.0 mg/l Kn and 0.25 mg/l NAA. Complete plantlets were obtained on the same medium or upon transfer of the regenerated plants. These plantlets were successfully established in the field after prehardening the plants (Mathur and Ahuja, 1991).

Picrorhiza kurroa Royle ex. Benth. a perennial herb of medicinal importance is a low, hairy herb with woody rhizome distributed to alpine Himalayas and grows in the inner ranges from Kashmir to Sikkim at an altitudinal range of 3000-5000m. A bitter extract obtained from the dried rhizomes of 3-4 years old plants has been widely used in traditional as well as modern systems of medicine as a stomachic, purgative, antiperiodic, and brain tonic and in dyspepsia and fever. The active principles of the extract are the three glycosides: Picroside I, Picroside II and Kutkoside. The plant has become endangered due to excessive collection in the Himalayan ranges. Clonal propagation was achieved through shoot tip culture supplemented with kinetin (3.0-5.0 mg/1). Addition of Indole-3 acetic acid (1.0 mg/1) to the kinetin showed marked improvement (Lal *et al.*, 1988). *In vitro* propagation method through forced axillary branching has been developed. Shoot cultures, initiated from stem cuttings of the plants collected from the Western Himalayas, multiplied at the rate of 36 fold every 4 weeks on a medium containing 1×10^{-6}m benzyladenine. Eighty-nine per cent of the *in vitro* multiplied shoots rooted on a medium containing 1×10^{-6}M NAA (Upadhyay *et al.*, 1989). According to them, plants were also regenerated from one mm shoot tips which should allow cryopreservation of this species. Micropropagation and *in vitro* conservation protocols have also been recently developed at NFPTCR (Sharma *et al.*, 1994).

Protocol for *in vitro* multiplication of Kuth (*Sassurea lappa*) was developed using Murashige and Skoog's medium containing benzylaminopurine and gibberallin allowing 3.5 fold shoot multi plication every three weeks (Arora & Bhojwani, 1989, Bhojwani *et al.*, 1989). Shoots also rooted on MS 0.5 m napthalene acetic

Table 2 . Medicinal/Aromatic plants of economic importance being worked at NFPTCR

Plant species	Active Principle	%	Medicinal use	Status
Coleus forskohlii	Forskolin	0.1	Treatment of glaucoma	Vulnerable
Coptis teeta	Berberin coptisin	7.7-8.9	Anti-inflammatory, anti-diabetic, anti-malarial	Endangered
Gentiana kurroo	Gentiopicroside	-	Improves apetite, use for leucoderma & syphilis	Threatened
Picrorhiza kurroa	Picroside I, II Kutkoside	3.0-4.0	Stomachic, brain tonic	Endangered
Podophyllum hexandrum	Podophyllatoxin	4.0	Anticancer	Endangered
Rauvolfia serpentina	Reserpine Ajmalicine	1 7-3.0	Used in hypertension insanity	Endangered
R. canescens	Reserpine Rauvolsine	-	Sedative, hypertensive skin ailments	Wild
Rheum emodi	Anthraquinones	2.0-3 0	Laxative, purgative	Vulnerable
R. moorcraftianum	,,	Purgative		
Saussurea lappa	Saussurine Essential oil	0.05 1.5	Antirheumatic, used in bronchial astham and skin diseases	Endangered
Tylophora indica	Tylophorine Tylophorinidine	0.42-0 46 0.009	Used in bronchitis rheumatic & goutypains	Vulnerable

acid with 90% efficiency. Shoot cultures stored at +5°C in the dark for 12 months without an intervening subculture survived with 100% viability.

Podophyllum hexandrum Royle. is highly important anticancerous plant species known for the production of podophyllotoxin. The Indian species is supposed to contain three times more podophyllotoxin than the American species (*Podophyllum peltatum*). Unfortunately, due to excessive and ruthless collection of its root and lack of any organised cultivation. *P. hexandrum* has become excessively threatened. *In vitro* multiplication of *Podophyllum hexandrum* via induction of somatic embryogenesis has been reported (Arumugam and Bhojwani, 1990). Callus derived from zygotic embryos on MS medium containing 2 NBA and 0.5 M IAA differentiated globular embryos. On this medium the globular embryos continued to multiply but failed to mature.

Further development of the embryos occurred when the sucrose was increased to 6% or the medium was supplemented with 1-10 μM NAA. Light and temperature higher than 25°C suppressed embryogenesis. The somatic embryos developed into plantlets on the basal medium. However, very efficient field establishment could not be attained. In National Plant Tissue Culture Repository, New Delhi, efforts to develop direct regeneration in *P. hexandrum* appears to be feasible. This will allow large multiplication of this valuable endangered species.

Propagation of Indian Rhubarh (Rheum emodi Wall.) was reported by Lal and Ahuja (1989) using shoot-tip explants, which gave rise to multiple shoots when cultured on M S medium supplemented with 2.0 mg/l 6-benzylaminopurine (BAP) and 1.0 mg/l indole-3 butyric acid. Shoot buds could also be induced from leaf explant using M S medium with 2.0 mg/l. BAP and 0.25 to 1.0 mg/l indole-3 acetic acid (IAA) or IBA. Rooting was initiated when shoots were placed on MS medium with 1.0 mg/l IBA, both regeneration procedures gave rise to healthy plantlets that were established in soils very well with 80% frequency after hardening.

Coleus forskohlii Briq., a vulnerable plant of high medicinal and economic importance, grows wild in the subtropical Himalayas, distributed from Kumaon hills to Nepal at 600-2300 m. The plant is the only natural source of forskolin, which is being used for the treatment of glaucoma, congestive cardiomyopathy, asthma and has anti-inflammatory properties. The procedure for *in vitro* multiplication of the species through axillary branching was worked out. Multiple shoots (12 shoots per 4 weeks) were obtained on MS supplemented with Kn and IAA which could be rooted on IAA- medium. These *in vitro* produced plants could be successfully established in soil. The forskolin content in tubers of these plants was 0.1%, which is same as that found in wild plants (Sharma *et al.*, 1991). *In vitro* propagation of the same species for forskolin synthesis has also been reported by Sen and Sharma, 1991. *In vitro* conservation work on the species has also been carried out and success achieved in prolonging the shelf life of shoot cultures (Sharma *et al.*, 1994).

Gentiana kurroo Royle. (Indian Gentian) an indigenous threatened plant, occurs as a perennial herb in the Himalayan region of India at an altitude of 1500-3300 m. The drug extracted from roots and rhizome is used to cure debility, urinary complaints, syphilis and leucoderma. Shoot multiplication of this species was achieved using shoots and nodal segments. Fifteen-fold shoot multiplication per 6 weeks was achieved on MS medium supplemented with BAP (8.9 m) and NAA (1.1 m). Rooting of these shoots was obtained on basal medium with 6% sucrose (Sharma *et al.*, 1993).

In vitro Conservation

Literature survey revealed that until now very limited scientific studies have been carried out on *in vitro* conservation with regard to medicinal plants (Table 1). Only in recent years systematic and well articulated programmes have been initiated. The National Bureau of Plant Genetic Resources has taken considerable lead in establishing Gene Bank and *in vitro* Repository. Realizing the needs and urgency of problems, concerted efforts were directed by National Plant Tissue Culture Repository (NFPTCR), operative at the National Bureau of Plant Genetic Resources to undertake extensive researches on the use of *in vitro*/cryopreservation technology. Establishment of aseptic cultures and efficient protocol for regeneration and multiplication of plants from desired species is the basic prerequisite for employing *in vitro* techniques for germplasm conservation. Therefore, the first stage was to develop procedure for rapid clonal multiplication of plant species under study.

The prominent endangered/vulnerable plants of medicinal and aromatic value are given in Table 2. Protocols for rapid propagation of *Coleus forskohlii, Gentiana kurroo, Picrorhiza kurroa, Podophyllum hexandrum, Rauvolfia serpentina. R. canescens, Saussurea lappa* and *Tylophora indica* were standardised (see Sharma *et al.*, 1994) and for *Rheum* species the protocols are currently being standardised. The major limitation of the work often is paucity of starting material in the threatened or endangered plant species, which is required to develop and work out the reproducible protocols. The cultures are initiated with limited available material (seeds/plant). The responding cultures are repeatedly subcultured on the responsive medium to build initial stock of cultures. Further, media modifications are then subjected to detailed testing to select optimum medium with respect to shoot growth, number of shoots etc. During the proliferation stage, axillary buds normally grow out from original explants and lateral shoots developed in the axils of leaves The *in vitro* generated micro shoots are then subcultured for rooting on semi- solid medium and plantlets are transferred to soil.

For transfer, the *in vitro* regenerated plantlets were removed from the culture tubes, washed thoroughly to remove the nutrient medium and transplanted to small pots with soil rite or soil and sand mixture (Sharma *et al.* 1991, 1992; Sharma & Chandel 1992 a, b).

For the initial period of transfer (2-3 weeks) potted plantlets were kept in the culture room condition and high humidity was maintained by covering the plants with polythene bags. Established plantlets were gradually acclimatized to out door conditions. Field transfer and ultimate survival of *in vitro* propagated plant species is crucial key step to achieve mass propagation. Often limited success has been reported in the literature, probably due to difficulty to mimic conditions conducive to proper growth particularly of Himalayan medicinal plant species.

There are two basic approaches to maintain germplasm *in vitro* : (a) growth limitation through minimal media, use of osmaticum, or using growth retardants etc. and (b) cryopreservation. In India, work is currently being carried out at NFPTCR on a range of species using *in vitro* technology, while cryogenic methods of long term storage have been investigated for several species (Kartha, 1984) and may provide the best solution to conserve endangered species. Shoot tip encapsulation and freeze preservation is yet another method found suitable for long term conservation. However, until routine methods for Cryopreservation are available, the alternative approach using growth limitation is simple, manageable and most appropriate. The advantage of this approach is that mostly the cultures can be visibly assessed for viability and can readily be brought back to normal culture conditions to produce plants on demand. Also multiplication of the plant system in present study was found to occur by enhanced axillary branching which is very crucial for employing tissue culture techniques for conservation of diversity as it ensures genetic stability.

Conservation experiments involve storage of *in vitro* cultures employing minimal media, use of osmoticum such as sorbitol or mannitol (2-5%) or incubation of *in vitro* cultures at low temperature. The results obtained so far showed that cultures could be maintained for more than 8-15 months depending on species and storage conditions (Table 3). A simple technique of replacing cotton plugs with polypropylene caps from the top of Borosil culture test tubes at normal culture conditions (25°C) could enhance the shelf life for over 8 months in ginger and turmeric (Balachandran *et al.*, 1990). Similarly, shelf life of *in vitro* cultures of *Rauvolfia serpentina, R. canescens* and *Coleus forskohlii* could be extended for over 8 months and upto 6 months in other culture systems using polypropylene caps. In contrast, the cultures covered with cotton plug remained viable for 2-5 months, which could be attributed to reduced evaporation of media in cultures covered with polypropylene caps.

Low temperature incubation appears to be highly promising as *in vitro* cultures of *Rauvolfia serpentina, Saussurea lappa* and *Tylophora indica* could be preserved for 15, 15 and 12 months at a temperature regime of 15°C, 4°C and 10°C, respectively. In case of *Saussurea lappa* and *Picrorhiza kurroa* also low temperature under dark conditions enhanced the subculture period.

Table 3 : *In vitro* multiplication and conservation responses of medicinal plant investigated at NFPTCR, NBPGR, Delhi.

Species	Shoot multiplication	Rooting	Field establishment	Storage period and conditions
Coleus forskohlii	+	+	+	18 M at 25°C
Gentiana kurroo	+	+	-	11 M at 4°C
Podophyllum hexandrum	+	+	-	NT
Picrorhiza kurroa	+	+	-	16 M at 10°C
Rauvolfia canescens	+	+	-	15 M at 15°C
R. serpentina	+	+	+	15 M at 15°C
Rheum emodi	+	+	-	20 M at 25°C
R. moorcraftianum	+	+	-	
Saussurea lappa	+	+	-	15 M at 4°C
Tylophora indica	+	+	-	12 M at 25°C & 10°C

In vitro cultures of *Rauvolfia* and *Saussurea* exhibited relatively slow growth with the inclusion of mannitol in the medium enabling maintenance of culture well over 9 months at normal culture conditions while over 15 months under low temperature. Experimentation is continued to assess the effect of osmoticum (Mannitol) under low temperature in other species also.

The stored *in vitro* cultures when put back on fresh medium for regrowth, showed shoot multiplication comparable to fresh cultures. Interestingly, some of the cultures showed better multiplication following short to medium term storage. Based on the technology developed at NFPTCR and the simple procedure being standardized. *In vitro* conservation offers a potential system for conserving endangered medicinal plants specially those in which the roots/rhizome/tuber or bulb constitute the source of biosynthetic products and are known for the active chemical constituents.

In vitro generated plants of *Rauvolfia serpentina, Tylophora indica*, and *Coleus forskohlii* on transfer to soil produced healthy plants and yielded as normal plants. Several successive crops were raised. For example healthy tubers harvested from *in vitro* generated plants of *Coleus forskohlii* yielded 0.1% forskohlin alkaloid on dry weight basis from well formed roots which corresponded very well with natural occurring plant populations (Sharma *et al.*, 1991).

In addition to above advancements, preliminary assessment have revealed a future scope for the production of artificial seeds through gel entrapment in *Coleus*. Their utilization as propagules for commercial plantings will be highly profitable. Once, such systems are tested on large scale, these may offer potential storage system for conservation of threatened and endangered plant species. It is gratifying that serious efforts are now being made for the conservation of threatened/endangered medicinal plant species. Several laboratories are presently engaged in developing suitable protocols; field gene banks and seed gene banks are also being establised apart from *in situ* conservation of germplasm diversity in medicinal plant species in natural habitats.

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Biotechnology and Genetic Improvement of Medicinal Plants

J.R.Sharma & Sushil Kumar

Central Institute of Medicinal & Aromatic Plants
Lucknow - 226 015

Introduction

DEVELOPMENT of superior strains/clones/varieties which manifest high degree of performance with respect to the productivity, quality and stability of the economic end-products in plants is the hallmark of crop genetic improvement. Such superior stocks/varieties are indeed the permanent and the cheapest source of changes in crop plants over generations and over the time and space. The genetical processes involved are very many suited to both environmental conditions and the target materials. Our intent, however, is not to elaborate and explicate here genetic principles and diverse breeding procedures and strategies adopted for crop improvement. But we do intend to project in brief its tremendous impact on revolutionarizing the agricultural crop production across the world with the sole objective to draw a corollary that similar revolution can be ushered in medicinal plants as well.

Many countries in the world harvested excessively increased yield of cereals by growing superior crop varieties during the past couple of decades or so resulting into a quantum jump in total production of grains. Such an amazingly phenomenal increase in crop yields caused ushering in an era of 'Green Revolution' in particularly South-East-Asian countries which harbours no illusion. In this backdrop of food plants, what is the state-of-art in medicinal plants? And, why and how can they be genetically manipulated, not only to enhance their end-products quantitatively but also qualitatively as well as adaptively to new areas and to newer

agencies? In the next few sections, we would like to capture the flavour of these very aspects and impress upon the gravity and exigency of exploiting the exciting capability of conventional plant breeding and modern biotechnology for genetic upgradation of medicinal plants under the herbal drug development programmes.

Need for Genetic Improvement of Medicinal Plants

In contrast to the food crops where yield ceiling is limited by genetic potential coupled with prevailing environment (Sprague, 1977); in medicinal plants, the production of raw materials is dependent upon a set of complex but integrated factors, namely genetic, environmental, socio-economic, marketing structures and all that. Direct collection of samples from natural resources and/or domestication of medicinal plants from wild stocks may perhaps maintain a sustained supply of raw materials for pharmaceutical industries for some period of time (Sharma, 1991 a & b). But this ostensibly suffers from three grave obstacles : One, the wild resources are not unlimited, hence cannot last for long; two, the production of raw materials through such collections cannot be assured; and three, materials sampled from natural forests are nearly always heterogenous thus adversely affecting the quality of herbal drugs and their efficacy. The simplest way to increase the yield (quantitative production) for assured supply may be to expand the area under cultivation. Crop husbandary too involving improved package of agronomic practices can raise the yield levels to the extent of genetic potential of the commercial bulk (domesticated wild/exotic stocks). However, the poor yield potential and heterogeneity of wild bulks brought under cultivation will not suffice the purpose of both quantitative and qualitative production of raw materials. Moreover, such plants under cultivation will have to compete with other food crops and also have to be cultivated under varying environments. Hence, increase in their production (quantitative yield) ought to be consummated by increase in productivity (yield per unit of area, time and input). But any way, no amount of agronomic maneuvering can help achieve the uniformity of the crop (to attract grower's acceptance) and quality of the yield (to elicit the preference of pharmaceutical concerns) both. This could however be achieved together with quantitative improvement only by expeditious and judicious genetic manipulation of these crops on permanent basis.

Thus, in nutshell, genetic improvement of medicinal plants is exigently necessitated to serve the following objectives:

(a) Fixing a high genetic ceiling by effecting permanent (heritable) improvement in wild/alien medicinal plants for higher productivity per plant, thus reducing the cost of cultivation and making them highly remunerative and cost-effective.

(b) Introducing impressive uniformity of crop under cultivation to attract grower's acceptance.

(c) Promoting the quality and homogeneity of herbal drugs to make them highly priced and saleable.

(d) Conserving the natural resources by restricting indiscriminate destruction by unscrupulus agencies. thus helping escape irreversible genetic erosion.

(e) Developing new crop cultivars well adapted to new areas of cultivation and also to newer agencies for their better exploitation.

(f) Converting them into a viable component of crop diversification under different cropping systems.

(g) Promoting the export potential of raw materials or even finished products and reducing or overstepping the import-gaps through increased production of quality produce.

Genetic Upgradation of Medicinal Plants at CIMAP

It is amazingly unfortunate that while upsurge in yield due to HYV leading to ushering in the era of 'Green Revolution' was witnessed in food crops (cf. Sharma 1994); virtually no serious attempts were ever made to genetically manipulate the herbal drug plants (Sharma, 1991 a). R & D works on medicinal plants commenced some four decades ago but mostly confined to chemistry, introduction and pharmacology. Only during the last one and a half decade or so, intensive work on genetic manipulation of these plants was initiated in right earnest in India (Sharma, 1990), though genetic manipulation of crop adaptation is an age-old process. Despite specific constraints imposed by their natural characteristic features, such as — importance of secondary metabolites more than or equal to that of primary metabolites, seasonal variation in the rate of biosynthesis of active principles, and variable and complex mode of reproduction, etc., some tangible successes have been achieved in developing genetically superior cultivars in some important medicinal plants over the years at CIMAP (Sharma, 1992). Besides, ample progress has also been made with respect to *in vitro* production of secondary metabolites, micropropagation of elite lines and endangered species, somatic hybridization, etc. through biotechnological approaches.

The current status of genetic improvement of medicinal plants through Mendelian and modern techniques both at CIMAP has been succinctly assembled here below.

(a) Genetic Improvement through Conventional Approaches

Six of the economically viable medicinal plants having national priority, namely opium poppy, periwinkle, Indian and Egyptian henbane, pyrethrum and quinghao were genetically manipulated through plant breeding leading to development of superior varieties (cf. Sharma 1990) (see Table 1). Besides, three other important medicinal plants, viz., psyllium, senna and chamomile were also lately subjected

Table 1: Genetically superior varieties of some medicinal plants released by CIMAP for commercial cultivation

	Crop	Cultivar released	% Superiority over the best check	Desirable end-product
1.	Opium poppy (*Papaver somniferum*)	*Shweta*	+57%	70° Latex (raw opium)
		Shyama	+64%	70° Latex (raw opium)
		Sanchita	+20%	Poppy-straw
			+25%	Seeds
			+50%	Straw-morphine
		Vivek	~	-do-
2	Indian henbane (*Hyoscyamus niger*)	*Aela*	+46% +109%	Biomass Crude drug
3	Egyptian henbane (*H. muticus*)	*HMT-1*	+15% +36%	Biomass Crude drug
		NP-41	~ +35%	Biomass Crude drug
4.	Pyrethrum (*Chrysanthemum cinerariaefolium*)	*Hansa*	+142%	Flowers
			+180%	Pyrethrins
5.	Periwinkle (*Catharanthus roseus*)	*Nirmal*	+20%	Leaves
			+195%	Roots
			+203%	Crude drug
6.	Quinghao (*Artemisia annua*)	*Asha*	+60%	Biomass
			+37%	Artemisinin

to genetic improvement under the first phase of our crop improvement programme at CIMAP. We may now enumerate successes in each of these crops.

1. Released Varieties

Intensive breeding efforts in the former six crops resulted into development of superior varieties which were subsequently released by CIMAP for commercial exploitation in different crops as under:

(i) ***Opium poppy***: This plant is a natural source of opium alkaloids, like morphine, codeine, papaverine and nascopine which are pharmaceutically unique (cf. Husain and Sharma, 1983). It is commercially grown mainly in Uttar Pradesh, Madhya Pradesh and Rajasthan States under the strict control of Narcotic Commission of India. Unlike the food growers having several improved varieties, opium poppy growers were cultivating age-old land races. They did not have any improved variety till 1984 when CIMAP released two improved gum harvest (GH) varieties — *Shweta* (with higher morphine concentration in latex) and *Shyama* (with

moderate morphine strength but high latex yield) giving 57% and 64% more yield of raw opium (70°), respectively than the local check.

While our efforts at CIMAP were still afoot to breed new GH varieties excelling *Shweta* and *Shyama*, global concern was shown since late eighties to do away with opium linked with menacing social abuses. One approach was the crop substitution of opium-yielding *P. somniferum* by non-opium species, *P. bracteatum*. However, this was not successful in India as the temperate plant *P. bracteatum* cannot be grown in sub-tropical climate where *P. somniferum* is cultivated. Another approach was to develop concentrated poppy-straw (CPS) varieties of *P. somniferum* with higher concentration of alkaloids in the poppy-straw (1/3 peduncle + capsule husk)where lancing to obtain latex is strictly prohibited. This is much prevalent in Turkey, USSR, France, Netherlands and Australia (Veselovskaya, 1976). Yet another approach — the ideal one — could be the genetic conversion of narcotic *P. somniferum* (latex $^+$) into non-narcotic one (latex$^-$) through massive mutagenesis programme and/or genetic engineering by blocking the pathway of latex-synthesis with the help of anti-sense technique or like. But this is, of course, a swim against the natural course of evolution, hence successes may not be guaranteed. Therefore, pending this last approach, we intensified our genetic improvement programme to develop CPS varieties. As a consequence, two CPS varieties — *Sanchita* and *Vivek* were recently released by CIMAP as a respite measure to combat social abuses associated with opium. Of these two *Sanchita* with 0.76% morphine in straw was found to excel even the world best recovery of 0.70% morphine reported from Tasmania (Australia). It is also a very good straw and seed yielder, hence can be adopted under cultivation. Indeed, the Narcotic Department has taken a lead to initiate its captive farming at appropriate sites in India.

(ii) ***Indian/black henbane*** An important national source of hyoscine (Scopolamine) and hyoscyamine - the tropane alkaloids, the Indian henbane is a good supplement to belladonna (Husain, 1993). It has limited cultivation in India, major part of herb is gathered from wild with poor alkaloid content and heterogenous quality. In order to harvest higher yields of biomass, and tropane alkaloids and to ensure uniform quality of produce under cultivation, the first superior variety - *Aela* was released by CIMAP during 1987 (cf. Sharma *et al.*, 1989), thus replacing the wild bulk for commercial cultivation. This registered 46% superiority in biomass and 109% in crude drug yields over the wild bulk (check). It is highly leafy and bears thin (less woody) branches possessing higher alkaloid content.

(iii) ***Egyptian henbane***: This was introduced in India from Egypt during late seventies Its biomass contains higher concentration of hyoscyamine than hyoscine (which is several-fold more important than the former in international market) vis-a-vis Indian henbane. However, being a smaller herb its cultivation is easier and more profitable in semi-arid zones than Indian henbane. To make its cultivation

successful, CIMAP released two varieties - a tetraploid *HMT-1* (Lavania and Srivastava, 1991) and a line *NP-41* (Tyagi, 1986) both around 35% superior for crude drug over the bulk check.

(iv) ***Pyrethrum***: This is a para-medicinal plant containing pyrethrins which is an eco-friendly biopesticide used as mosquito repellant (Singh and Sharma, 1989). This is an introduction from Kenya, grown as commercial bulk under temperate climate of Kashmir valley (J&K) and Palni hills (T.N.). In order to make its cultivation cost-effective, yielding higher amount of dry flowers, with high pyrethrins concentration, per unit area, CIMAP released a superior variety, *Hansa* during 1985. The variety is exceedingly superior over the commercial bulk (check) for both flowers (+142%) and pyrethrins (+180%) yields under Palni hills conditions.

(v) ***Periwinkle***: A wonder drug plant containing vinblastine and vincristine in the leaves and ajmalicine in roots, the leaf alkaloids being potentially effective as anticancer agents (monocytic leukaemia, lymphosarcoma, carcinoma of breast, lungs and testes), and the root alkaloid as hypotensive agent (Gjerstad, 1965; Farnsworth, 1962). It is omnipresent, but prefers tropical and subtropical climatic conditions. Its commercial cultivation is however very limited in India, though it has now attracted world-wide attention because of its pharmaceutical significance. Considering this exigency, CIMAP released a genetically improved variety, *Nirmal* for extending its profitable cultivation. Although var. *Nirmal* is at par with local bulk for alkaloid concentration in both leaves and roots, it is 20, 195 and 203% better than bulk for the yields of leaves, roots and crude drug, respectively. It is also tolerant to die-back disease, hence enjoys good establishment in the field.

(vi) ***Quinghao***: A well-known anti-malarial Chinese drug plant, quinghao was recently introduced in India. Its active constituent - artemisinin has been found to be very effective against chloroquin-resistant strains of malarial parasite, more so against cerebral malaria (brain fever) (cf. Husain, 1983). Its bulk cultivation is now established in Kashmir valley. With a view to improving its biomass yield and artemisinin content, a superior variety - *Asha* was released by CIMAP during 1988 for commercial cultivation. This variety, basically an improved population gives 60% more biomass and 37% more artemisinin content than the introduced bulk material (check). It grows well in subtropical climate also, but does not set seeds.

In addition to these released varieties, under the germplasm enhancement programme, many more promising lines (see Table 2) have also been identified and evaluated over years under initial evaluation trials (IET), bench scale trials (BST), pilot scale trials (PST) and yield optimization trials (YOT) at CIMAP (Annonymous, 1992, 1993). They are now in pipeline to be graduated shortly for commercial cultivation

Table 2: Genetically superior elite strains of medicinal plants in pipeline for release by CIMAP

	Crop	Elite strains	% Superiority over the best check	Desirable end-product
1.	Isabgol (Psyllium) (*Plantago ovata*)	M-22 (*Niharika*)	+170%	Seeds Pods + Leaves
2.	Senna (*Cassia angustifolia*)	S-121 (*Suphala*)	+70%	Blue oil
3.	German chamomile (*Chamomilla recutita*)	M 70-1 (*Vallary*)	+200% + 60%	Flower heads
4.	Opium poppy (*Papaver somniferum*)	DR-3 (*Shakti*)	+50%	Latex (raw opium)
		Mass-2B (*Sampada*)	+25%	-do-
5	Indian henbane (*Hyoscyamus niger*)	Rb (*Aekla*)	+50% +70%	Biomass Crude drug

2. Elite Strains in Pipeline

Varietal improvement programme was also initiated in three new medicinal plants, viz., psyllium, senna and German chamomile, and further intensified in opium poppy and Indian henbane. As a result, superior strains were identified in these crops as elaborated below (see Table 2):

(i) ***Psyllium***: This plant, which is of Mediterranean origin, is a source of natural laxative - the property present in its seeds and husk. In India, it is cultivated mainly in Gujarat and to a limited extent in adjoining Rajasthan and Haryana States (Anonymous 1976, Modi *et al.*, 1974). As the seeds and husk both are of substantial export-potential, the area under this crop must be expanded. With this objective, breeding for improved varieties of psyllium, especially adapted to north Indian plains commenced at CIMAP a few years back. Now we have identified a superior strain - M 22 (*Niharika*) which under north Indian conditions gives seed yield as good, if not more, as harvested in western India. In fact, it registered 170% more seed yield under a pilot scale trial at Lucknow than the check var. Gujarat Isabgol-1 (which is perhaps not well suited to north Indian conditions).

(ii) ***Senna***: This is also an exotic plant native to Somalia and South Arabia. It was introduced in India long back by Arab traders. Its leaves and pods contain sennosides which have laxative/purgative property. Its cultivation is confined mainly in Tamil Nadu State. However, next to psyllium, senna (leaves + pods) is the biggest export commodity of India; it is, therefore, imperative to expand its cultivation in newer areas so as to increase its production for export. As such, CIMAP started improvement of genetic stocks by developing superior strains of senna suitable for north Indian plains (Lal *et al.*, 1992) and now a strain—S-121

(*Suphala*) has been identified, which registered 70% superiority over the commercial bulk (check) for leaves + pods yield in pilot scale trial at Lucknow. It is much more rich in sennosides also than the check.

(iii) ***Chamomile***: A native of southern and eastern Europe, chamomile is the natural source of an essential oil, known in commerce as 'Blue Oil' which has wide curative effects and uses (e.g. anti-inflammatory and softening effects on the digestive tract when used internally, and applied externally for treatment of hemorrhoids and inflammations of the opthalmic mucosa and uterus). Its flower-heads too are used as drug (Salamon, 1992). Its bulk stock/population was introduced in India from Germany, USA and Bulgaria for commercial cultivation. But its yield potential (oil and flower-heads) was poor, hence CIMAP took up its genetic improvement programme through mutation breeding which resulted into identification of a superior strain - M701 (*Vallary*) (Lal *et al*., 1993). In a semi-commercial pilot scale trial, it registered 200% superiority over the best check - German Bulk for oil yield and 60% for flower-head yield. Its very deep blue oil is indicative of superior quality It grows well even in alkaline soil (PH9).

(iv) ***Opium poppy***: Though our emphasis has now shifted from developing GH varieties to CPS varieties, it will perhaps take some time to discontinue cultivation of latex varieties. The simple reason is that opium itself is the biggest foreign exchange earner for India. In view of this, simultaneously armed with CPS varieties (*Sanchia* and *Vivek*), CIMAP continued breeding for still petter GH varieties. As a consequence, we have now identified a dwarf recombinant - DR-3 (*Shakti*) and a downy mildew resistant strain—Mass-2B (*Sampada*) giving 50% and 25%, respectively more latex yield than the best check (*Shyama*) under pilot scale trials.

(v) ***Indian henbane***: Owing to non-condusive plant-type characterized by initial prostrate branching restricting population pressure per unit area and overlapping leaf-arrangement rendering the plant physiologically less efficient, a genetic rectification of plant-type through mutagenesis was initiated during early eighties. A superior mutant variety *Alae* was released earlier. But its plant-frame remained the same, though primary branches became thin and number of leaves and tropane alkaloid content both increased, hence superior on an over all basis. Concurrent with polygenic changes (Sharma *et al*., 1992), an unbranched (Ub) mutant was also detected in M_2 generation. It was an unique genotype because a dozen traits were changed at one stroke: it was unbranched (with single main stem, permitting higher population density, hence higher biomass per unit area) with leaves thick, and large, arranged in non-overlapping fashion (hence physiologically most efficient plant-type), thus completely altering the plant-frame to be quite desirable. Higher crude drug content than even *Aela*, provided a premium to this Ub mutant. But seed-setting was very poor (though seeds are not the economic product, they are used essentially for sowing the crop). Hence, selection for high seed-setting began over generations

While selecting for high seed-setting in Ub progenies, a restricted branching (Rb) segregant with 2-4 erect branches appearing from the top (unlike prostrate branches appearing from the bottom in the original/normal plant) with better seed-setting but quite akin to parental Ub mutant for all other features, was identified in later generation. Further rigorous selection for seed-setting brought this Rb mutant at par with the normal. In view of its physiologically most efficient plant type, high herb yield and high alkaloid concentration, coupled with near-normal seed-setting, the Rb mutant (*Aekla*) is now ready for commercial cultivation.

Thus, from the foregoing statements, it is apparent that tangible successes have been achieved by CIMAP in developing and releasing a number of genetically superior varieties/strains in different medicinal plants through conventional plant breeding approaches

(b) Genetic Manipulation through Biotechnological Approaches

In addition to conventional plant breeding approaches, since the last nearly one decade, CIMAP has made a distinct headway in genetic manipulation of medicinal plants through biotechnological approaches also, particularly cell/tissue culture techniques (see Table 3) in the following areas:

1. Micropropagation

Tissue culture is routinely used for *in vitro* propagation and conservation of various plant species (cf Nand Lal and Ahuja 1993). Such multiplication of medicinal plants has been successfully accomplished in several species. Following aspects of micropropagation have been worked out at experimental level, advancing to the field conditions:

(i) ***Direct regeneration from explant.*** In appropriate culture-media (varying protocols) using shoot tip and auxillary bud explant in *Valeriana wallichii* (Mathur *et al.*, 1988), shoot tip and leaf explant in *Rheum emodi* (Nand Lal and Ahuja 1989), shoot tip in *Picrorrhiza kurroa* (Ahuja *et al.*, 1988), and stem-node explant in *Duboisia myoporoides* (Kukreja and Mathur 1985), high frequency of plant regeneration has been achieved and plantlets were successfully transferred to the field conditions.

(ii) ***Regeneration from calli***: Calli were induced from petiole explants in *V. wallichii* (Mathur and Ahuja 1991), and from segment of flower head explant in *Allium govanianum* (Kukreja 1992) wherefrom complete plantlets were regenerated and subsequently transferred to the field following the standard hardening procedures.

(iii) ***Regeneration through somatic embryos***: Somatic embryogenesis was successfully induced from callus culture in *Aconitum heterophyllum* (Giri *et al.*, 1993) and *Solanum sarrachoides* (Banerjee *et al.*, 1994) and from protoplast culture in *Hyoscyamus muticus* (Giri & Ahuja 1990). Complete plantlets were formed and

Table 3: Genetic manipulation of medicinal plants through biotechnological approaches at CIMAP

	Medicinal plant species	Techniques employed	State of success/target achieved
1.	*Picrorhiza kurroa*	Direct regeneration from explants	Plantlets regenerated and transferred to field for micropropagation and conservation of germplasm
2.	*Glycyrrhiza glabra*		
3.	*Duboisia myoporoides*		
4	*Valariana wallichii*	(i) Direct regeneration from explants	
5.	*Rheum emodi*	(ii) Regeneration from calli	
6.	*Allium govanianum*	Regeneration from calli	
7	*Aconitum heterophyllum*	Regeneration through somatic embryos	
8.	*Solanum sarrachoides*		
9.	*Hyoscyamus muticus*		
10.	*Withania somnifera*	Agrobacterium mediated genetic transformation	The best strain - A_4 of *Agrobacterium* efficient for transformation identified
11.	*Atropa belladonna*		The cheap and indigenously marketed antibiotic-Cephalaxin identified as a substitute for imported cefotaxime
12.	*Panax quinquefolium*	*In vitro* production of secondary metabolites	The presence of saponin in callus and medium both recorded
13.	*Rauvolfia serpentina*	(i) Direct regeneration from explants (ii)Regeneration from calli	Establishment of complete tetraploid plant from the induced tetraploid plant with 4*n* shoots but 2*n* roots
14.	*Hyoscyamus albus*	Somatic hybridization with *H. muticus*	Somatic hybrids with *H. muticus* produced

established in soil in all the target materials. These results could be important in developmental regulation of morphogenesis in plants.

(iv) ***Establishment of induced autotetraploid of rauvolfia***: Colchi-autotetraploid shoots were induced in *R. serpentina*, but the roots (the planting material) stayed diploid. Therefore, propagation and multiplication of complete autotetraploid plants was conventionally not possible (seeds were sterile). But complete autotetraploid plantlets were regenerated from the induced tetraploid shoot, following tissue culture technique either through auxillary bud elongation or via multiple shoot formation on supplemented MS medium (Mathur *et al.*, 1987). This is of considerable consequence for commercial exploitation of tetraploid *Rauvolfia* and other such plants.

(v) Improved Methods for Rapid Micropropagation: Nand Lal and Ahuja (1993) demonstrated that use of agar-solidified culture medium for micropropagation is not as cost-effective as the use of liquid medium. This is because of more labour needed, high cost of agar and wastage of nutrients in the medium at the end of subcultures in case of gelled media, besides poor growth and proliferation rates of cultures. With the aim of devising an economical procedure in terms of media, time and space they examined the performance of liquid static and shake cultures vis- a-vis the agar-solidified culture in *Rheum emodi* and found the best results of multiple shoot formation on using the liquid shake culture (120 RPM). This caused at least 50% reduction in medium requirement and 37.5% in time and led to 1.5-2.2 fold increase in growth and multiplication rate in comparison to agar solidified cultures. Besides, liquid culture-raised plantlets also facilitated easy transplantation and ensured 90-95% survival of plantlets on transfer to potting mix in glass house.

Kukreja (1994) also suggested an improved method of high frequency of plant regeneration through multiple shoot formation from nodal explant in *Glycyrrhiza glabra*.

2. Morphogenesis

Rapid clonal propagation through tissue culture of elite lines where sexual or asexual propagation is a limiting factor, is most frequently exploited either through direct organogenesis in cultured tissues or through an intervening callus phase. The former route, i.e. direct organogenesis is preferred over the latter as it ensures genetic uniformity and retention of morphogenetic potential. Different explants can be used for clonal propagation, but they must possess morphogenetic potential. Kukreja *et al.*, (1988) obtained successful plant regeneration via organogenesis from foliar explants of *Duboisia myoporoides* — an important source of tropane alkaloids. Based on the success, the process was upscaled and 50 trees were established in field. The procedure outlined by them has the potential for the production of thousands of trees per explant per annum.

Beside the effects of explant, role of antibiotics to supplement the culture media, either for selection of drug-resistant cell lines to serve as 'marker' in the *in vitro* genetic manipulation programme or for overcoming problems of microbial contamination of culture media, has been studied by several workers for better understanding of possible biochemical and molecular events underlying morphogenesis *in vitro*. Some antibiotics are toxic while others are stimulatory for cell growth and enhanced shoot differentiation in callus cultures. Kukreja *et al.*, (1987) demonstrated that certain antibiotics, like chloramphenicol exhibited stimulatory effect in shoot-bud organogenesis at levels upto 150 mg/l in *Hyoscyamus muticus* callus. A 6-10 fold increase in shoot-bud primordia-formation was observed at 50 mg/l. Not only this, a two-fold increase in total tropane alkaloid content was also observed at this level which supported optimal shoot morphogenesis This, obviously highlights the possible role of antibiotics in differentiation and morphogenesis *in vitro* and their potential use for production of secondary metabolites at the callus and cell levels.

3. Agrobacterium Mediated Genetic Transformation

Hairy root culture is the most frequently used method for genetic transformation, i.e. transfer and expression of foreign genes (genetic material DNA) in plants The technique involves co-culture of explants with *Agrobacterium rhizogenes* (with root inducing Ri plasmid) for specified duration followed by transfer to a suitable medium supplemented with antibiotics to eliminate bacteria for plant regeneration. The success of genetic transformation depends largely upon the efficiency of *A rhizogenes* strain employed and the type and dose of antibiotics used in the culture media

In an important medicinal plant, *Withania somnifera*, Banerjee *et al*., (1994) analysed the specificity of the bacterium strain and the frequency of transformation events. They found the best transformation ability and growth of the hairy roots of the strain A_4 of *A rhizogenes* They also observed the presence of withafrin-A in the media as well as in the hairy roots of 10 weeks old cultures.

Besides, Sultana and Ahuja (1993) studied the effect of many bacteriocidal antibiotics used in hairy root culture of *Atropa belladonna* for genetic transformation. They found an indigenously marketed cheap drug - *Cephalexin* as a better alternative to the imported *Cefotaxine* heretofore being extensively used in genetic transformation experiments.

4. *In vitro* Production of Secondary Metabolites

The first successful commercial production of Shikonin from cell of the plant, *Lithospermum erythrorhizon* by M/s Mitsui Petrochem Ltd in Japan opened the new possibilities and renewed enthusiam for production of secondary metabolites, like alkaloids, saponins, flavonoides, etc. from cell cultures of medicinal plants

Banerjee *et al.*, (1993) in collaboration with Bose Institute, Calcutta identified fruit as the best explant or tissue source of *Solanum sarrachoides* for *in vitro* production of solasodine - a nitrogen analogue of diosgenin. The amount of solasodine so obtained even without optimization studies, was higher than that reported in several other species of the genus *Solanum*.

Besides, Mathur *et al.* (1994) also demonstrated *in vitro* production of ginsenoside — an adaptogenic and stimulatory saponin from 25-day old callus and cell suspension cultures of *Panax quinquefolium*. They also observed the typical segmoidal growth pattern of its callus and cell suspension tissues over a 35-day culture cycle on modified MS medium.

5. Somatic Hybridization

Development of parasexual (somatic) hybrids is an exciting possibility of remote hybridization at interspecific and even at intergeneric level for *inter se* transfer of novel genes inter se. The two species of *Hyoscyamus*, viz. *H. muticus* and *H. albus* are wide apart in chromosome number (2n = 28 and 68, respectively). The former contains more hyoscyamine than hyoscine and is susceptible to aphids and viruses. In contrast, the latter has high scopolamine (hyoscine) in its roots and is resistant to insects and stresses. But owing to pre-zygotic incompatibility, sexual hybridization between these two species was not successful. However, Liaq-ur-Rahman *et al.* (1994) were able to develop parasexual hybrids from fused protoplast of these two species and thus combine characters of interest across species. The somatic hybrid plants showed intermediate morphological characters with 82-120 chromosomes (modal number being 96). They produced 33-78% viable pollen grains and set viable seeds upon selfing and backcrossing with either parents.

Thus, a good deal of success has been achieved in genetic manipulation of medicinal plants through biotechnological tools, especially tissue/cell culture techniques at CIMAP. But it would require considerable time and input to reach to commercial scale. Nevertheless, it has shown the path definitively.

Blue Print and the Future Vision

As could be visualized, all these medicinal plants enumerated hitherto have direct bearing in modern system of medicine, because the major/active chemical constituents and their biological activities in these plants have very well been established. The literature also abounds with numerous reports on basic genetic studies in these crops, which we purposely skipped to save time and space. But this is by no means all that is the need of the hour. An array of herbal drug plants which play unique roles in traditional systems of medicine where not the specialty chemicals but the whole crude drug (the compound mixture) is important, is yet to be subjected to organized cultivation and intensive genetic acceleration. Therefore, our future vision underlies the conservation and genetic amelioration of myriads of untouched

or scarcely manipulated herbal flora which nature has so generously bestowed upon us. Fortunately, the recent biotechnological advancement has crumbled all the major bottlenecks, hence rapid successes might be assured. The final product, i.e. an improved cultivar arising from genetic enhancement of germplasm of these plants might confer many advantages:

(1) It is a permanent genetic restructuring (stable/heritable over generations) of the germplasm lines.

(2) It is the cheapest way of germplasm enhancement, hence the most cost-effective component of superior cultivation technology.

(3) The product is no doubt environmentally clean and bio-friendly.

(4) The product may fit into varying cropping systems, such as intercropping, crop-rotation, agro-forestry, etc.

However, a close and adequate monitoring is expeditious while subjecting the target material to genetic manipulation. For instance,

(a) Whether the final product commensurate with the prevailing demand of pharmaceutical market for its qualitative profile.

(b) Whether the new strain/variety matches well with the environment of its cultivation without any loss of quantitative and qualitative performance. In other words, whether the product is appropriately buffered from genotype x environment interaction.

(c) Whether the equilibrium of the biotic and abiotic stresses is properly maintained under cultivation.

These strategies on genetic manipulation of herbal drug plants will set the human health care system on sound scientific footing. Not only this, the conventional/Mendelian plant breeding coupled with modern and sophisticated biotechnological tools may soon become the harbinger of 'Green Drug Revolution' (GDR) vis-a-vis 'Green Revolution' (GR) in food crops. However, as against the latter (GR) which has often been regarded as an agronomic splendour but societal disaster and is browning/waning fast (Harlan, 1977); the former (GDR) might overcome social imbalances as HYV's evolved in medicinal plants may fit well into both conservative (subsistance) as well as opportunistic (modern) agricultural systems.

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Improvement on Medicinal Plants Cultivation in West Bengal Hills

R.P.Nandi

Directorate of Cinchona & Other Medicinal Plants
Govt. of West Bengal
Mungpoo - 734313, Darjeeling, India

Introduction

THE active principles which are found in plants are generally secondary metabolites, the physiological activities of which have rendered them important as potential drugs and for related uses. The biosynthesis of these active compounds, although controlled genetically, is affected strongly by environmental influences[1]. As a result, there are fluctuations in the concentrations and quantities of the secondary metabolites such as alkaloids, glycosides and steroids etc. There are scattered reports on detailed studies regarding the effect of environment on medicinal plants. Poor informations are available regarding the physiological studies in relation to developmental growth of medicinal plants. Systematic studies have been reported by different authors in different crops like *Cinchona*[2,3,4], *Cephaelis*[5], *Rauvolfia*[6,7,8], *Catharanthus*[9] and *Datura*[10,11]. Scientific cultivation of *Dioscorea* and extraction of their active principles have been reported by Mehta & Staba[12] and Nandi & Chatterjee[13,14]. Panda[15] analysed the agronomical aspects of *Costus speciosus* plants. The developmental aspects of *Digitalis* have been analysed by Nandi & Chatterjee[16,17] and also by others[18,19,20]. The present report is an attempt to improve medicinal plants cultivation in West Bengal hills.

Material & Methods

Seedlings of alkaloid yielding plants like *Cinchona ledgeriana, Cephaelis ipecacuanha, Solanum khasianum, Rauvolfia serpentina, Catharanthus roseus, Datura innoxia, Datura stramonium, Atropa belladonna* and *Hyoscyamus niger*; steroidal sapogenin yielding plants like *Dioscorea composita*, Dioscorea floribunda, *Dioscorea prazeri* and *Costus speciosus* and cardiac glycoside yielding plants like *Digitalis lanata* and *Digitalis purpurea* were used as experimental materials. Experiments were conducted in randomised block design. in experimental nurseries at different altitudes. Active principles of different plants were assayed following I.P.[21] for *Cinchona* and *Cephaelis*; Nandi & Chatterjee[22] for *Rauvolfia*; Janot *et al.* for *Catharanthus*; Scieder[11] for *Hyoscyamus, Datura* and *Atropa*; Selvaraj[24] for *Dioscorea* and *Costus* and A.O.A.C.[25] for *Digitalis*. Nitrogenous fractions viz total nitrogen (TN), protein nitrogen (PN) and soluble nitrogen (SN) contents were estimated following the method of Vogel[26]. Estimation of reducing sugar (R-CHO) and non-reducing sugar (NR- CHO) contents were done according to the method described by Panda[15]. Post harvest incubation and histochemical studies on *Costus* and *Dioscorea* were analysed following the method described by Nandi *et al.*[27]

Quantitative data on stem growth, leaf formation, flower/ fruit formation and formation of different active principles were analysed systematically at intervals of 15 - 30 days according different species and percentage increase or decrease of different growth and biochemical functions during some predetermined interval of time, together with the formation of active principles in different plants were worked out. Such data were collected during vegetative, reproductive, and post-reproductive stage of development[28] in all the plants species under study.

Experiments were conducted by removing the flower buds (de-budding) and fruits (de-fruiting) to find out the role of reproduction in the synthesis of active principles. In long-day (18L + 8D) experiments, additional light was supplied to the plants being 100 Fc for 15 days and 30 days. In other experimental studies, the plants were grown in different altitudes to find out the best suitability of altitudinal zones for their cultivation. Growth hormones like gibberellic acid (GA_3) and mineral nutrients like Mg and Mn were applied through leaves in different concentrations. The results of effective optimum doses have been included in the report.

Experimental Results

Experimental results obtained from *Cinchona* and *Cephaelis* growing at different elevations revealed that *Cinchona* prefers high range of elevation for maximum bark yield and alkaloid formation. In *Cephaelis*, though the alkaloid synthesis is favoured at higher altitude but the root yield is maximum in lower elevations (Tables 1,2). *Rauvolfia serpentina* can favourably be cultivated at lower altitude when alkaloid content is also high but in *Catharanthus* the alkaloid content is

maximum at medium elevation and the herbage yield at lower elevation (Table 3). Studies on altitudinal effect on *Datura* revealed that the alkaloid formation is favoured at lower altitude whereas the formation of the same was favoured at higher altitude in *Atropa, Hyoscyamus* and *Solanum* (Table 4). Variations in diosgenin formation in *Dioscorea* and *Costus* have been shown in Table 5. Synthesis of diosgenin was more pronounced in plants cultivated at high altitudes but the production of biomass was generally high in lower elevations. In *Digitalis*, the cultivation of plants at higher altitude favoured the production of biomass as well as the glycoside formation in both the species. Glycoside formation was of lower magnitude at lower elevations (Table 6).

Table 1 — Effect of altitude on bark yield and alkaloid content in *C.ledgeriana.*

Altitude (M)	Bark yield kg/plant		% alkaloid	
	Root	Stem	Quinine	Quinidine
100	1.08	0.40	4.21(2.66)	0.16(0.06)
500	1.10	0.42	4.32(2.73)	0.17(0.06)
1000	1.12	0.48	4.85(2.97)	0.18(0.09)
1500	1 25	0.62	4.91(3.12)	0.18(0.11)
2000	1.11	0.41	4.65(2.60)	0.17(0 06)

Figures within the parenthesis is % alkaloid of stem bark.

Table 2 — Effect of altitude on root yield and alkaloid content in *C.ipecacuanha* root

Altitude (M)	Root yield/ plant(gm)	Total alkaloid (%)	Emetine (% of TA)
50	9.1	2.4 to 2.5	45 to 50
100	8.5	2.6 to 2.7	50 to 52
500	8.0	2.9 to 3.2	50 to 55

Table 3 — Effect of altitude on root yield and alkaloid content in *Rauvolfia* and *Catharanthus*

Species	Altitude (M)	Root yield/ plant(gm)	Total alkaloid (%)
	100	58.5	2.22
R.serpentina	500	47.2	2.01
	1000	42.3	1.95
C.roseus	100	67.9	0.97
	500	62.5	1.11
	1000	58.3	1.01

Table 4 — Altitudinal variations of alkaloid formation in *Datura, Atropa, Hyoscyamus* and *Solanum*

Species	Altitude (M)			
	50	500	1000	1500
A.belladonna	0.13	0.15	0.16	0.18
H.niger	0.76	0.80	0.85	0.88
D.innoxia	0.46	0.32	0.30	0.26
D.stramonium	0.49	0.35	0.32	0.28
S.khasianum	0.92	0.98	1.10	1.15

Table 5 — Effect of altitude on diosgenin and biomass production in *Dioscorea* and *Costus*

Species	Altitude(M)	Biomass(kg/A)	Diosgenin(%)
D.composita	50	5500	3.00
	500	4800	3.50
D.floribunda	50	4550	3.54
	500	4100	3.79
D.prazeri	50	2000	2.00
	500	1100	2.21
C.speciosus	50	2250	1.21
	500	1200	1.85

Table 6: Effect of altitude on biomass and glycoside content in *Digitalis*

Species	Altitude (M)	Yield/ha (Qntl)	Total glycoside (%)
	100	27.5	0.6 to 0.8
D.purpurea	500	52.5	0.7 to 0.9
	1000	75.0	1.0 to 1.5
D.lanata	100	12.0	0.4 to 0.6
	500	21.0	0.5 to 0.6
	1000	30.0	0.8 to 0.9

The assay of alkaloid content in roots of *Rauvolfia* and *Catharanthus* and in leaves of *Datura, Hyoscyamus* and *Atropa* was done during vegetative, reproductive and post-reproductive stages of development (Table 7). Developmental stages were distinguished from each other in respect of quantum of flowers and fruits

formation per plants. A positive accumulation of alkaloid formation occurred in all stages of development of *Rauvolfia* and *Catharanthus* whereas a negetive accumulation of the same was represented in *Datura, Atropa* and *Hyoscyamus* during post-reproductive stage of development. Maximum accumulation of alkaloid was noted in all the species during reproductive stage of development when there also occurred a maximum rate of flower formation. Rate of formation of total glycoside (TG) in both rosette (R) and axial (A) leaves as well as flower formation during different developmental stages of *Digitalis* have been shown in Table 8. The TG content in A-leaves gradually increased upto initiation of reproductive stage of development i.e. late bolting stage and its rate of formation in these leaves became highest when rate of flower formation was maximum. In R-leaves, though TG formation increased upto initiation of reproductive stage, its rate became highest in these leaves before initiation of flower took place on the bolted axis. Insteroidal sapogenin yielding plants, the diosgenin synthesis in *Dioscorea* occurred during all the stages of development, maximum rate of synthesis being noted during vegetative stage of development. In*Costus*, diosgenin synthesis increased upto reproductive stage of development and there after declines. Maximum rate of synthesis of diosgenin was noted during reproductive stage when the rate of flower formation was also maximum in this plant species (Table 9).

The effect of removal of flower buds (de-budding) and removal of fruits (de-fruiting) on growth and active principle contents in different medicinal plants have been shown in Table 10. De- budding and de-fruiting operations accelareted leaf formation in all the species under study except *Rauvolfia* where there is a decrease in laminar area formation. Maximum increase in leaf area formation was

Table 7 — Alkaloid formation in relation to reproductive function in alkaloid yielding plants.

Species	% increase(+)or decrease (-) during		
	Vegetative	Reproductive	Post-reproductive
R. serpentina	FL -	+ 70.06	+ 55.66
	TA + 28.62	+ 36.53	+ 29.65
C.roseus	FL -	+ 750.00	+ 60.08
	TA + 12.60	+ 25.51	+ 18.35
D.innoxia	FL -	+ 330.00	+ 51.00
	TA + 13.75	+ 17.90	- 6.97
A.belladonna	FL -	+ 280.00	+ 34.70
	TA + 15.45	+ 22.83	- 7.05
H.niger	FL -	+ 190.50	+ 21.30
	TA + 19.51	+ 31.32	- 4.25

Table 8 — Rate of glycoside formation in relation to flowering in *Digitalis*

Species	Parameters	% increase(+)or decrease (-) during			
		Vegetative	Bolting	Reproductive	Post-reproductive
	Fl formation	0	0	+ 380.45	+ 5.10
D.lanata	TG R-leaf	+ 10.52	+ 15.16	+ 2.43	- 5.12
	TG A-leaf	0	+ 8.10	+ 12.28	- 4.31
	Fl formation	0	0	+ 320.51	+ 10.22
D.purpurea	TG R-leaf	+ 17.20	+ 22.55	+ 9.53	- 4.32
	TG A-leaf	0	+ 15.19	+ 19.33	- 4.15

Table 9 — Rate of diosgenin formation in relation to flowering in *Dioscorea* and *Costus*

Species	Parameters	% increase(+) or decrease(-)during		
		Vegetative	Reproductive	Post-reproductive
	Fl formation	0	+ 1142.85	+ 9.62
D.composita	Diosgenin	+ 2.50	+ 2.25	+ 2.06
	Fl formation	0	+ 62.08	+ 3.61
IC. speciosus	Diosgenin	+ 12.46	+ 19.95	- 10.31

noted in *Digitalis lanata*. The de-budding operation decreased the linear growth in all the alkaloid yielding plants (except *Rauvolfia*) and cardiac glycoside yielding plants where the active principle synthesis was augmented in all the species by both the operations.

The effect of light and GA_3 on the formation of active principle in different medicinal plants have been shown in Table 11. Long-day treatments and foliar application of GA_3 augmented the biogenesis of active principle in all the plants in a considerable way. Maximum augmentation was noted in *Rauvolfia serpentina* plants treated with 30 long day photoperiodic cycles and in *Datura innoxia* plants treated with 200 ppm gibberellic acid.

Analysis of different physiological and biochemical functions during different developmental stages in *Atropa, Datura* and *Hyoscyamus* has been shown in Table 12. It is revealed that long day exposure to the plants increased the total alkaloid synthesis during vegetative stage of development. Extension growth (EG) was found to be positively correlated with total alkaloid synthesis during vegetative stage. Alkaloid synthesis was also found to be correlated with flower formation during reproductive stage of development of the plants. Analysis of biochemical fractions revealed that during vegetative stage all the biochemical fractions along

Table 10 — Effect of removal of reproductive units on growth and active principle formation

	% increase(+)or decrease(-) over control					
	Removal of flower buds			Removal of fruits		
Species	Leaf growth	Linear growth	Active principles	Leaf growth	Linear growth	Active principles
D.stramonium	+ 9.9	- 19.2	+ 43.3	+ 4.2	+ 13.4	+ 21.9
A.belladonna	+ 28 1	- 21.3	+ 29.3	+ 15.2	+ 7.3	+ 17.1
H.niger	+ 45.7	- 18.6	+ 33.6	+ 18.2	+ 5.0	+ 19.6
R.serpentina	- 5.9	+ 3.8	+ 17.6	- 3.2	- 4.2	+ 11.5
C.roseus	+ 25.8	- 14.9	+ 19.6	+ 30.1	- 2.3	+ 9.2
C.ledgeriana	+ 15.8	- 6.2	+ 29.5	+ 7.1	- 2.2	+ 21.3
C.ipecacuanha	+ 10.4	- 8.2	+ 17.2	+ 6.3	- 3.6	+ 11.1
D.lanata	+ 98.6	- 50.0	+ 17.2	+ 78.9	+ 30.6	+ 10.3
D.purpurea	+ 38.4	- 29.5	+ 12.6	+ 14.6	+ 22.6	+ 9.3
C.speciosus	+ 26.0	+ 22.6	+ 32.1	+ 14.2	+ 11.2	+ 9.7
D composita	+ 22.6	+ 18.3	+ 12.5	+ 8.3	+ 11.7	+ 6.2
D.prazeri	+ 12.6	+ 10 1	+ 9.3	+ 6.6	+ 7.1	+ 4.2
D floribunda	+ 18.6	+ 14.2	+ 11.3	+ 8.5	+ 7.9	+ 5.1

Table 11 — Effect of longday and GA_3 on active principle formation

	% increase(+) or decrease(-)over control	
Species	30 LDPPC	GA(200 ppm)
A.belladonna	+ 17.00	+ 8.22
D.innoxia	+ 21.13	+ 22.16
D.stramonium	+ 21.59	+ 9.56
H.niger	+ 19.78	+ 11.58
C.roseus	+ 3.22	+ 6.72
R serpentina	+ 36.10	+ 11.39
D.lanata	+ 15.67	+ 14.05
D.purpurea	+ 14.85	+ 11.16
D.composita	+ 13.90	+ 13.63
D floribunda	+ 14.32	+ 12.52
D.prazeri	+ 15.36	+ 11.32
C.speciosus	+ 21.73	+ 16.35

with the TA content increased when treated with long day photoperiodic cycles, a maximum increase being noted in SN content. During reproductive stage, decrease of TN and PN content remained associated with increase of SN and TA contents. Such study when examined during post reproductive stage revealed that TN and PN contents increased during this stage with the decrease of SN and TA contents. Results of effects of long day on *Dioscorea* have been shown in Table - 13. It is revealed that extension growth gradually decreased with the advancement of stages of growth along with the synthesis of diosgenin. Maximum extension growth as well as diosgenin synthesis was revealed during vegetative stage of development. It is also observed that during all the developmental stage, there occurred a positive accumulation of all the biochemical fractions. The rate of diosgenin and R-CHO synthesis was maximum during vegetative stage and then gradually declined whereas the rate of NR-CHO content gradually increased showing maximum

Table 12: Physiological and biochemical correlation in alkaloid yielding plants treated 30 LDPPC

Parameters	% increase(+) or decrease(-) over control		
	Vegetative	Reproductive	Post-reproductive
A.belladonna			
EG	+ 35.22	+ 7.52	+ 2.16
FI	0	+ 69.93	+ 13.11
TN	+ 5.32	- 1.95	+ 2.47
SN	+ 9.84	+ 15.47	- 8.22
PN	+ 8.10	- 15.33	+ 9.53
TA	+ 10.25	+ 17.00	- 3.21
D.stramonium			
EG	+ 28.19	+ 6 52	+ 2.13
FI	0	+ 122.82	+ 12.55
TN	+ 17.16	- 2.84	+ 5.38
SN	+ 30.81	+ 17.00	- 10.69
PN	+ 16.42	- 14.52	+ 11.50
TA	+ 18.84	+ 21.59	- 6.05
H.niger			
EG	+ 41.39	+ 8.22	+ 2.11
FI	0	+ 98.52	+ 7..59
TN	+ 13.19	- 2.15	+ 4.13
SN	+ 23.52	+ 21 88	- 7.89
PN	+ 12.16	+ 14.18	+ 7.33
TA	+ 14.53	+ 19.78	- 7.42

Table 13 — Effect of 30 LDPPC on the pattern of changes of functional parameters in *Dioscorea composita*

	% increase(+) or decrease(-)over control		
Parameters	Vegetative	Reproductive	Post-reproductive
EG	+ 121.11	+ 22.80	+ 8.73
FI	0	+ 1340.00	+ 48.18
TN	+ 8.00	+ 2.25	+ 3.01
R-CHO	+ 10.12	+ 8.33	+ 8.02
NR-CHO	+ 2.31	+ 3.56	+ 5.22
Diosgenin	+ 3.90	+ 3.78	+ 3.14

increase during post-reproductive stage of development. Table 14 shows the glycoside content in *Digitalis* increased due to application of GA_3 during vegetative stage with the reduction of R-leaf formation. It is to be noted that with the increase of TG content during reproductive stage of development, the rate of extension growth, A-leaf formation and flower formation increased. Thus, during reproductive stage, a positive correlation between the rates of increase of extension growth, A- leaf formation and formation of flowers and TG content could be shown. GA_3 treatment increased all the biochemical fractions during vegetative stage. During reproductive stage, TN and NR- CHO contents decreased which was accompanied by an increase of R- CHO and TG contents. During post-reproductive stage, TG and R-CHO contents decreased with the increase of NR-CHO.

The results of application of Mg and Mn in different medicinal plants have been shown in Table 15. Both Mg and Mn increased dry weight accumulation and alkaloid synthesis in all the species. A reduction in linear growth was observed in *Rauvolfia* and *Catharanthus*. Plants treated with Mn revealed higher potentialities than Mg in synthesising alkaloid. Dry weight accumulation and formation of alkaloid were increased in all the treatments and a close relationship could be established between these two biochemical entities. The foliar application of mineral nutrients like Mg,Mn and B showed pronounced effects on augmentation of tuber growth and diosgenin formation in *Dioscorea* and *Costus* (Table 16). All treatments augmented diosgenin formation as well as tuber yield. Maximum tuberisation was noted in plants treated with Mg + Mn + B (10 mg/1) whereas synthesis of diosgenin was favoured in plants treated with Mg + Mn (10 mg/1) treatment.

Tables 17 and 18 showed the histochemical analysis of *Costus* and *Dioscorea* respectively. In *Costus speciosus*, there revealed a distinct correlation between the microscopic characters of the starch grains and the corresponding diosgenin content. The starch grains in the older rhizomes with the high diosgenin content were

Table 14 — Effect of GA-200 ppm on pattern of changes of functional parameters in *D. purpurea*

	% increase(+) or decrease(-)over control		
Parameters	Vegetative	Reproductive	Post-reproductive
R-leaf	- 14.31	0	0
A-leaf	0	+ 183.61	- 26.81
EG	0	+ 201.74	+ 15.22
Fl	0	+ 109.33	+ 12.75
TN	+ 3.01	- 2.11	+ 1.07
R-CHO	+ 4.20	+ 5.35	- 2.68
NR-CHO	+ 1.19	- 3.69	+ 2.42
TG	+ 8.63	+ 11.15	- 2.81

Table 15 — Effect of Mg and Mn on growth and alkaloid formation in alkaloid yielding plants

	% increase(+) or decrease(-) over control					
Species	Linear growth		Dry weight		Total alkaloid	
	Mg	Mn	Mg	Mn	Mg	Mn
C.ledgeriana	+ 3.5	+ 3.6	+ 34.2	+ 47.5	+ 44.1	+ 66.2
C.ipecacuanha	+ 12.7	- 11.3	+ 14.8	+ 16.1	+ 20.6	+ 25.3
R.serpentina	- 17.4	- 5.1	+ 18.6	+ 21.6	+ 21.1	+ 30.0
C.roseus	- 29.7	- 12.2	+ 25.3	+ 39.8	+ 22.5	+ 54.1
D.stramonium	+ 14.2	+ 9.7	+ 9.3	+ 11.5	+ 11.3	+ 22.6
A.belladonna	+ 12.4	+ 7.8	+ 11.5	+ 26.5	+ 17.9	+ 31.2
H.niger	+ 10.6	+ 6.2	+ 19.1	+ 32.4	+ 21.7	+ 29.3

Table 16 — Effect of mineral nutrients on tuberisation and diosgenin formation in *Dioscorea* and *Costus*

Treatments	Kg tuber/plant			% Diosgenin		
(10 mg/1)	DC	DF	CS	DC	DF	CS
Mg	0.62	0.55	0.66	4.68	5.46	2.22
Mn	0.61	0.53	0.64	4.60	5.32	2.15
B	0.60	0.51	0.60	4.33	5.06	2.13
Mg + B	0.61	0.53	0.61	4.20	4.49	2.10
Mg + Mn	0.62	0.54	0.65	4 69	5.48	2.25
Mn + B	0.61	0.52	0.60	4.18	4.40	2.06
Mg + Mn + B	0.64	0.58	0.69	4.31	5.00	2.16
Control	0.55	0.50	0.52	3.30	3.70	1.85

DC = *D. composita*; DF = *D.floribunda*; CS=*C.speciosus*

Table 17 — Distribution of starch grains in relation to age and diosgenin content in *Costus speciosus*

			% of starch grains in:		
Age (yr)	Diosgenin (%)	Frequency of starch grains	Cortex	Ground	Around vascular bundle
0.5	0.70	96	100	92	26
1.0	1.50	79	90	66	40
1.5	1.80	65	77	53	64
2.0	2.30	58	57	46	75

Table 18 — Distribution of starch grains in relation to age and diosgenin in *Dioscorea composita*

Age (yr)	Diosgenin (%)	% occurrence of starch grains/vision field				
		Very small round	Small round	Large round triangular	Large elliptical	Large lense shaped
1.0	2.01	98	2	0	0	0
2.0	2.71	30	65	5	1	0
3.0	2.79	5	8	70	12	5
4.0	3.84	2	5	10	20	83

larger and oval, being restricted in number; whereas in younger rhizomes with low diosgenin content, the starch grains were abundant, small and spheroidal in shape. A tendency of accumulation of starch grains surrounding the vascular bundles with the increase of age and diosgenin content could also be established. With the advancement of age in *Dioscorea composita*, the diosgenin synthesis increased along which there occurred a change in the shape and size of starch grains. In one year old plants, the diosgenin content in the tuber is low when the number of very small round starch grains is maximum. The shape of the starch grains passes some transitional stages (triangular and elliptical) and become lens shaped at the age of the 4 year old crop when diosgenin content is also high. A tendency of accumulation of starch grains around vascular bundles with the increase of diosgenin in *Costus speciosus* could also be established (Table 19). The post-harvest incubation with 2,4-D and Na-acetate established the relationship of starch grain size, number and orientation with diosgenin content. Treatments which induce higher diosgenin content, showed lesser dimension and frequency of starch grains.

Discussion

Experimental studies distinctly established an altitude effect on formation of active principles; *Cinchona, Cephaelis, Atropa* and *Hyoscyamus* prefer medium range of

Table 19 — Change in starch grain characterisation and diosgenin content of *C.speciosus* influenced by post-harvest treatment.

Chemicals (ppm)	Diosgenin (%)	Starch grain characteristics: Frequency Ground	Around VB	Number/ cell	Size (µm)
Control	1.56	77	58	19	28 ×10
2,4-D 10(24h)	2.19	57	35	15	30 ×11
2,4-D 10(48h)	3.36	24	21	9	34 ×12
2,4-D 10(60h)	3.19	29	20	9	33 ×12
Na-acetate 50(48h)	3.79	16	14	6	35 ×14
Na-acetate 50(60h)	3.23	20	16	7	33 ×11

Figures within the perenthesis indicate incubation period.

elevations whereas plants like *Rauvolfia, Catharanthus, Datura, Solanum* and *Dioscorea* prefer lower elevations. In *Datura, Catharanthus* and *Solanum*, though the active principle content was maximum in plants at higher elevations but biomass out put increased in plants growing in lower altitudes.

The alkaloid biogenesis was of lesser magnitude during vegetative stage when the plants had actively followed physiological and biochemical functions. Reproductive stage of development recording minimum growth rate, permitted the biogenesis of alkaloid to proceed most effectively. Thus it could be stated that the rate of synthesis of alkaloid/glycoside remained associated with the formation of reproductive refluxes. During later stages of development, active principle content declined due to mobolisation to the developing fruits[29].

Additional light augmented the synthesis of active principles and longer photoperiods consistently revealed a higher active principle content as compared to shorter photoperiods. The augmentation of the synthesis of active principles with LDPPC have also been mentioned by other authors.[30-33] However, a demonstration of quantitative affects of LDPPC on enhancement of active principle synthesis in conjunction with augmentation of different growth and biochemical functions, as revealed, has not been reported so far.

De-budding and de-fruiting operations enhanced the longivity of the plants. The removal of flower buds enhanced the leaf growth and at the same time retarded the linear growth and senescence was sufficiently delayed confirming the findings of Gupta[34] in *Nicotiana plumbaginifolia*. Developing flowers and fruits are considered to be effective mobilising cehtres of different metabolites in plants and an early removal of these units could be said to prevent depletion, resulting in modification of synthesis of active principles in these plants.

Foliar application of different mineral nutrients was found to regulate growth and active principle synthesis. Augmentation of active principle synthesis by Mg and Mn have been reported by the author[35,36] in earlier studies and in the present study it was found that active principle content increased along with the increase of dry matter accumulation and a close relationship could be established between these two biochemical entities.

Histochemical studies highlightened the ready detection of good clones. An inverse relationship between size of starch grains and diosgenin content in steroidal drug yielding plants, opened up the possibilities of forecasting the establishment of an economic plantation. Post-harvest incubation increased the diosgenin yield with a possibility of increasing factory efficiency by augmenting production.

It is seen that TN and PN contents gradually increased during vegetative stage of development and thereafter declined, SN increased till the reproductive stage of development. The increase of SN could be said to remain associated with increased synthesis of alkaloids. It might be indicated that TN and PN contents during vegetative stage could primarily remain associated with increased growth efficiency till the termination of the said stage. It has been reported that synthesis of alkaloids takes place by bringing together the derivatives of carbohydrates from the leaves and ammonia via roots. According to Mothes[37], gearing of these two biochemical entities into the mechanism of plant respiration during metabolism is must for alkaloid synthesis. The alkaloids have been considered to rise through decomposition of proteins[38,39] and the present study revealed the existence of an agreement between the phenomena of decrease of TN and PN and increase of TA and SN in alkaloid yielding plants. In steroidal drug yielding plants, there occurred an increase on TN and R-CHO with the increase of diosgenin content. The NR-CHO content gradually decreased with the increase of diosgenin and has established an agreement between the phenomena of increase of TN,R-CHO and diosgenin and decrease of NR-CHO content. In cardiac glycoside yielding plants an agreement has been established between the phenomena of decrease of TN and NR-CHO and increase of total glycoside and R-CHO contents. It might be said that increasing accumulation of R-CHO, mainly derived from accumulating photosynthates, could serve one of the ingredients to be channellised into the formation of glycoside in this study.

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*Improvement of Medicinal and Aromatic Plants through Induced Mutations

S.N.Kak* & B.L.Kaul

Regional Research Laboratory,
Canal Road, Jammu - 180 001

Introduction

GENETIC improvement of crop plants depends upon the available gene pool and its manipulation. A finite gene pool can only yield a finite response (Simmonds, 1983). While Plant introduction represents the convetional source and recombination following hybridisation the usual source of genetic variation, induced mutation provides a potential alternative to both induction and hybridisation because mutations are ultimate source of genetic variability. Natural variation is simply the product of spontaneous mutations, moulded by recombination and sieved through natural selection. Induced mutations not only stimulate the spontaneous variation but produce many new variations and those that have been lost from natural population during the course of domestication and cultivation.

The direct use of mutations in the improvement of medicinal and aromatic plants lies in their being a valuable supplementary tool to plant breeding, particularly when it is desired to improve one or two easily identifiable traits in an otherwise good plant type. The main advantage of applying this technique of improvement to these crops is that the basic genotype of the variety is only slightly altered as compared to hybridisation.

*Revised and updated

Moreover induced mutagenesis have been shown to widen genetic base for quantitative characters commonly referred as micro mutations (Gregory 1955, 1956). Thus this approach of improvement got a filip because most of the important characters contributing the yield have been shown to be quantitative in nature. Since then concerted efforts of mutation breeders have resulted in the release of more than 200 improved crop varieties (Sigurbjornesson and Micke, 1974). The techniques adopted in the improvement of medicinal and essential oil bearing plants are basically same as that of the cereal crop but differ only in details keeping in view required end product.

In case of vegetatively propagated medicinal and aromatic plants natural variation for most of the commerically important characters are more or less fixed, thus conventional breeding methods of improvement can not be of much consequence. Induced mutation breeding is particularly significant in such crop plants (Broertjes & Van Harten, 1978). This unique situation prompted Sigurbjornesson and Micke (1975) to highlight the use of induced mutations in vegetatively propagated plants as the most promising technique of improvement.

Medicinal and essential oil bearing plants have a very short history of domestication and cultivation compared to cereal crops. Systematic efforts to use induced mutations as a tool for the improvement of these crops have started in the very recent past. Nevertheless results obtained so far by these studies have been very encouraging.

MEDICINAL PLANTS

Solanum spp

Solasodine bearing *Solanum khasianum* has been intensely studied for the genetic upgrading reflected in the form of improvement of its steroid content, berry yield and elimination of its thorns on the plant which hampers in harvesting the berries. The use of radiations have lead to induction and isolation of curved spine mutant genotype having improved berry yield per plant together with higher alkaloid content (Bhatt, 1972, Bhatt and Heble and Heble, 1978). In their extensive studies on the species Kaul and Zutshi (1974, 1977) and Kaul *et al.*, (1976) were successful in inducing variability in a number of agromically important characters of the crop through induced mutagenesis. These studies resulted in the isolation and development of new mutant genotype (RRL - 20/2) exhibiting improvement in berry yield and solasodine content. Some mutant strains also have been reported having reduced size and frequency of spines on the plant together with improvement in the fresh berry yield (Chuhan *et al.*, 1975; Chuhan 1978, Kaul and Zutshi, 1976). Chuhan and Ravindran (1979) reported isolation of mutant plant type having long styled flowers coupled with higher berry yield per plant.

Ram (1989) in his extensive studies on the genetic improvement of the crop, was able to generate significant variability in some of the important agronomic characters followed by isolation of a number of improved mutant genotypes.

Another solasodine containing species of genus *Solanum* is *S.laciniatum* which has been subjected to induced mutation studies with a view of genetic improvement of the plant. An early flowering mutant together with a higher yield of berries per plant and glycoalkaloid content has been isolated (Szabady and Tetenyi, 1972).

Dioscorea spp.

Radiations have been used in the genetic upgrading of the diosgenin containing *Dioscorea* spp. Martin *et al.*, (1974) was successful in increasing tuber formation and disogenin content in *D. alata* and *D. bulbifera* by this technique. Similarly Pal and Sharma (1974) isolated improved strains of *D. deltoidea* as a result of exposure of tubers to radiations. A new type of sapogenin bearing mutant clone of *D. bulbifera* has been reported (Pal and Sharma, 1979). In *D. floribunda* two year old crop raised from radiation exposed tubers exhibited both very high and very low disogenin bearing clones (Banerji *et al.*, 1984).

In another diosgenin bearing plant, *Costus speciosus* ionizing radiation has been successfully employed in generating variability in economic characters leading to the isolation of improved genotypes of the plant (Laxmi *et al.*, 1980).

Atropa

Both ionizing radiations and chemical mutagens have been employed effectively in generating variability in agronomically important characters of the crop (Choudhary and Kaul, 1973; 1977; Kaul and Choudhary, 1973). Gamma-rays have also been used in developing mutant plant types having altered morphological characters (Chirghita *et al.*, 1981).

Datura spp.

Genetic improvement through the use of induced mutations technique has been successful in *Datura metel* (Kaul *et al.*, 1973; 1976; Sobti & Kaul 1982). It has been observed that very low doses of radiation have stimulatory effect in various agronomically important characters of the plant (Kaul *et al.*, 1976).

Papaver

Radiation as well as chemical mutagens have been employed for induction and isolation of improved genotypes of morphine and thebaine alkaloid bearing *Papaver*. Michalski (1969) and Invanova (1972) have been able to develop improved morphine containing genotypes of *Papaver somniferum* exhibiting improved alkaloid content in them. The problem of seed shattering in *P. bracteatum* has been a

major constraint in its development as a source of alkaloid. To overcome this problem Levy (1985) has been successful in isolating shattering resistant mutant genotype of the crop.

AROMATIC PLANTS

Mentha spp.

A number of essential oil bearing species of mints have great industrial importance and as such considerable work on their genetic improvement through induced mutations have been undertaken

(i) Mentha arvensis var *piperances*

Popularly known as Japanese mint is the only natural source of aroma chemical, menthol. Basic radiobiological studies which are prerequsite for mutation breeding studies were first conducted by Ono (1971, 1972, 1974) and also by Ono and Ikeds (1969). In India where the crop is grown on a commercial scale average oil and menthol content in the oil of the crop has been lowest resulting less renunierative to cultivators. As such efforts to improve existing clones has been started in the very recent past To this end Mittal *et al.*, (1972) observed that radiation treatment of the crop were able to improve some of the agronomically important characters without affecting some of the desirable characters of the crop. Similarly Kaul and Kak (1974) were able to induce considerable variability in various agronomically important characters as a result of gamma ray treatments. These extensive studies led them to isolate and develop a number of mutant clones exhibiting improvement in some of the imporant agronomic characters (Kak & Kaul 1977, 1978, 1980).

(ii) M. piperata var *vulgaris*

The oil obtained from this crop is important in cosmetic and perfumery industry. Number of researchers have attempted to breed genetically superior clones of the crop through induced mutagenesis. The stimulatory effect of low radiation doses on various important agronomic characters have been observed by various workers (Berezina and Korneva, 1964; Ozola and Eisenberga, 1966). Sadowska (1974, 1975) observed that various doses of ionizing radiation treatment showed progressive improvement in both quantitative and qualitative characters of the crop. Contrary to this, Gupta (1966) reported that radiation exposure resulted in reduced biomass yield. Murray (1969) in his classical work on inducing and isolating *Verticillium* resistant clone of the crop was successful in evolving the disease resistant strain, when all other techniques of breeding of resistant variety had failed. This disease resistant strain of *M. piperata* had all the desired agronomic and flavour charactersitic of the oil of parental clones. The mutant clone has completely replaced the susceptible clone in the United States (Murray, 1971). Recently CIMAP has isolated and developed a mutant clone of the crop having about 30% more biomass yield than the parent clone (Singh *et al.*, 1982).

M. citrata

This species of genus *Mentha* is a good source of two aroma chemicals namely linalool and linalyl acetate. Genetically superior clones have been obtained by Todd and Murray (1968) and Murray (1969) through the use of induced mutation technique. Similarly Kak and Kaul (1977, 1976,1988) were able to isolate and develop a number of mutant clones having improved content of linalool and/or linalyl acetate in their oils by using ionizing radiation technique, for the genetic upgrading of the crop.

Mentha spicata

The essential oil of the species is a rich source of aroma chemical Carvone. To bring about genetic improvement in the crop Kak and Kaul (1981) were able to isolate and develop fourteen mutant clones of the crop which included a mutant clone having almost double the amount of carvone in its oil compared to parent clone. Earlier Green (1975) utilized induced mutagenesis technique in isolating disease resistant mutant clone of the crop.

Some of the other economically important mint species where induced mutation studies for genetic improvement have been conducted, are *M. cardiaca* (Horner & Melouk, 1976) and Karaswa *et al.* (1977) who recorded novel types of essential oils in mutant clones of *M. rotundifolia*.

***Cymbopogon* spp.**

Genus *Cymbopogon* is an important industrial source of wide array of essential oils and aroma chemicals. Many of the species, cultivars of the genus are at present under commercial cultivation. Use of radiation and radiochemicals in the imrovement of some of the commercially important *Cymbopogon* species were stated much earlier.

Kapoor and Datta (1967) found that ionizing radiation treatment of dormant slips in some of the economically important *Cymbopogon* species resulted in improvement of tillering which overall resulted in improvement of biomass yield of the plants. In support of this Gupta (1969), Gupta and Kapoor (1970) found that induced mutation studies in *C. martinii* resulted not only in improvement of tillering and biomass yield but also essential oil content of the crop.

Choudhary *et al.* (1976) found that radiations can be successfully employed in creating necessary variability in various agronomically important characters of Genus *Cymbopogon* . Similarly Choudhary and Kaul (1979) were successful in reducing undersirable constituent, methyl-eugenol in the oil of *C.flexosus* which otherwise has been suggested a good source of citronella oil; A geranyl rich mutant clone of *C. nardus* var. *confertiflorus* was developed through induced mutation studies (Choudhary *et al.*, 1980; Kak *et al.*, 1981). Similarly Kak and Kaul (1982, 1983, 1984) were able to generate sufficient variability in a number of agronomically important characters of some of the important *Cymbopogon* species. Ganguly

et al. (1979) used chemical mutagens for breeding of improved clones of Java citronella. Subjecting the seeds of *C. martinii*, to various doses of ionizing radiations & from the treated plants so evolved it was observed by Srivastava and Tyagi (1986) that while some radiation doses resulted in improvement in herbage yield other doses resulted in improvement in essential oil quality. Similarly Sharma *et al.* (1987) were able to evolve improved citral rich clone of *C. pendulus*.

***Rosa* spp.**

Genus Rosa is a large polymorphic essential oil bearing genus. The rose oil has very important place in Perfumery industry. Thus efforts have been made very early in developing elite clones of essential oil bearing roses. Chan (1966) and Streitberg (1967) were successful in isolating several useful mutant clones of oil bearing roses. Gupta (1966), Gupta and Shukla (1969) observed that radiation treatments not only affected flower shape and size but also essential oil content of the plants. Heslot (1966, 1968) recorded desirable changes in the essential oil content in some essential oil bearing roses as a result of ionizing radiation treatments. These findings drew support from the work of Lata (1973) and Lata and Gupta (1971) who were able to isolate some mutant clones of roses having favourable qualitative and quantitative changes in their essential oils. Wakajima (1970, 1973) also was able to obtain pure non-chimeric promising mutant clones of some roses by induced mutation technique. On the contrary Kaicker and Swarup (1972) could not come to a defnite conclusion about the utility of induced mutation studies in the improvement of roses. However Raev (1985) was able to isolate and develop some essential oil rich clones with desirable oil characteristic as a result of induced mutation.

Geranium

The earlier mutation studies in *Pelargonium* spp. were mainly concerned with establishing some of the basic radiobiological parameters such as germination, survival and growth rate for breeding through ionizing radiations treatments (Bergann and Bergann, 1959. Potsch, 1964). Chikovani and Chitya (1975) reported through their studies on improvement of essential oil content in some clones of *Pelargonium* when subjected to ionizing radiation treatment. Similarly considerable variability in some of the important agronomic characters of the crop were generated by Skirvin and Janick (1976) through ionizing radiation treatment. The crop is very much affected by root - rot, and nematode attack. To overcome this problem a resistant variety of the crop has been evolved (Doraswamy and Sundram, 1978).

Davana Oil

This essential oil is obtained from the flower heads and fresh herbage of *Artemisia pallens*. This low volume and high value crop has a exquisite and delicate aroma and as such is used in high grade perfumes and cosmetic preparations. The oil is also used in the prepartion of many beverages and eatables (Kak, 1993).

In view of the fact that oil is exclusively produced in India, there is no significant genetic variability in various accessions of the crop (Farooq *et al.*, 1990). Efforts are underway for improvement of the crop through induced mutation studies (Farooq *et al.*, 1990; Kak *et al.* 1994).

Olive

The olive oil is obtained from *Olea europea* and is mainly grown in Mediterranean countries. The plant is an important source of essential oil. The main breeding objective in Olive has been to produce spur type growth habit mutants of various cultivars to be used in dense plantations and enable fruit picking.

A number of mutant types of the crop have been isolated having desired agronomical characters (Donini *et al.*, 1975; Donini 1976). A novel technique of improving essential oils reflected in the form of improvement of main constiuents of the oil and their odour, whereby essential oils and aroma chemicals were directly exposed to ionizing radiations has been established. There radiation exposures resulted in the improvement in quality and olfactory characters of the oil (Kak and Kaul, 1991).

Results achieved so far in the improvement of medicinal and aromatic plants through induced mutations studies have been quite significant, and it also indicates the vast scope that technique offers for the genetic improvement of these crops in short span of time compared to conventional breeding methods.

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Chemistry of Some Medicinal Mushrooms

V. George, Om V. Singh and Asha S. Koshy

Phytochemistry Division,
Tropical Botanic Garden and Research Institute
Pacha-Palode, Thiruvananthapuram 695 562

THE nutritional and medicinal properties of mushrooms have been recognised for long in human history. Ancient Chinese courts valued the mushroom *Lentinus edodes* for its elixir and aphrodisiac properties and it is said that the growing sites of these mushrooms were guarded by soldiers. The wood rotting fungus, *Ganoderma lucidum* has been an ingredient of several Chinese medicinal preparations. In China, this drug is known as Lin Zhi Kao. The medicinal properties of mushrooms were known to the ancient Indian physicians. Charaka classified mushrooms under vegetables - *Saka varga*, while Susruta classified them under flowers - *Udbhita*. Some authors such as Gordon Wasson believe that the divine drink of immortality of the Vedas - *Soma*, constituted the mushroom *Amanita muscaria*. The hallucinogenic properties of certain species of *Psilocybe*, *Inocybe* etc. were known to man for long and are still collected and consumed for their psychotomimetic effect. These hallucinogenic mushrooms were used for religious and recreational purposes by some Mexican and Siberian tribes. Apart from their medicinal and nutritional properties, the colourful mushrooms find mention in several folklores and fairy tales. In Kerala, mushroom shaped tomb stones, known in Malayalam as *Kudakallu*, are found in several ancient burial sites. Thus mushrooms served a special role in all aspects of human activity - food, medicine, religion, recreation, literature, folklore, customs and traditions.

Mushrooms, in a short span of life time, synthesis a wide variety of primary and secondary metabolites. Secondary metabolism in fungi is an enigmatic process influenced by environmental and other external stress factors. In the growth and

development of the fungus, secondary metabolism occurs after a period of hyphal growth has taken place. The mycelia may excrete the secondary metabolites to the surroundings or store them in different parts of the fungal body. The medicinal or edible qualities of the mushroom is decided by the nature and action of the metabolites produced by the mushroom. Hence a thorough and detailed chemical and biological investigation is necessary to ascertain the medicinal or toxic properties of a fungus.

Though the medicinal properties of mushrooms were recognised for a long time, it is only in recent years that the fungi were subjected to detailed chemical and pharmacological investigations. Out of a large number of primary and secondary metabolites 'isolated from mushrooms, only a limited number have been studied for their biological activities. Most of the work in recent years have been concentrated on the biologically active macromolecules from fungi. In this paper, recent developments in the chemistry and pharmacology of some selected mushrooms with medicinal potential are reviewed.

Agaricus

Among the members of *Agaricus*, *A. bisporus* is widely cultivated and consumed. Some members of the genus possess medicinal properties.

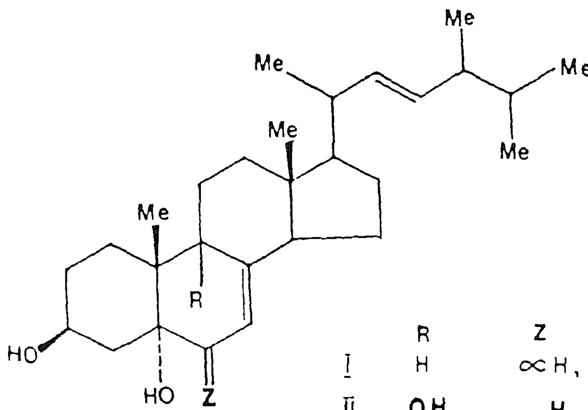
A pharmaceutical preparation containing nucleic acid components as neoplasm inhibitors was isolated from *Agaricus* mushrooms. The antitumour activity of the nucleic acid components against Sarcoma-180 was demonstrated. The preparation consisted of nucleic acid components 100 mg, Mg stearate 250 mg and lactose 24.65 mg mixed and encapsulated by gelatin[1].

Agaricus blazei

A. blazei, known as Himematsutake in Japanese is a home remedy having many physiological activities[2]. A glycoprotein, m.wt. 1.5×10^4, $[\alpha]^{25}_D$ - 23°, having 50.2% sugar and 43.3% protein isolated from *A. blazei* showed antitumour activity[3]. An extract of this fungus having 5.54% ash, 43.19% crude protein, 3.73% crude lipids, 6.01% fibres was shown to improve liver functions in CCl_4 treated rats[4]. A β-D-glucan polysaccharide isolated from *A. blazei* showed immunostimulative antitumour activity[5]. From the fruit bodies of this mushroom a protein linked (1A6) β-D-glucan was isolated. This glycoprotein exhibited antitumour activity[6].

Three ergosterol derivatives (**I**), (**II**) and (**III**) isolated from *A. blazei* showed antitumour properties[7].

Water insoluble polysaccharides were obtained from the fruit bodies of *A blazei* by successive extraction of hot water extracted fruit bodies with (1) 1% ammonium oxalate solution, (2) 5% NaOH solution, (3) 20% NaOH solution and (4) 5% LiCl-dimethyl acetamide solution. The polysaccharide-protein complex



	R	Z
I	H	∝H, β-OMe
II	OH	H, -OH
III	OH	O

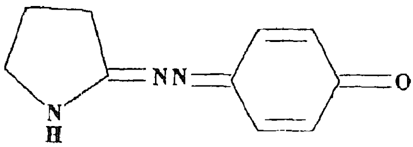
obtained from 5% NaOH solution showed antitumour activity against Sarcoma - 180 in mice and it had 50.2% carbohydrate and 43.3% protein. The carbohydrate portion consisted of (1Ä6)-β- D-glucan and its protein part was rich in aspartic acid, glutamic acid, alanine, leucine and proline. A high antitumour activity was found in another xyloglucan-protein complex obtained from the same 5% NaOH solution[8].

Agaricus campestris

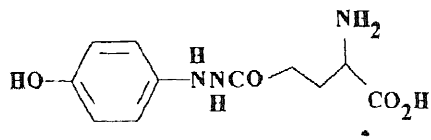
From the hot water extract of the mycelial culture of the *A. campestris* a glycoprotein fraction, showing antitumour activity against Sarcoma-180 in ICR mice, was isolated. This fraction consisted of a polysaccharide moiety (45%) and a protein moiety. The polysaccharide moiety contained mannose (42%), glucose (25.5%), xylose (16.6%), fucose and galactose. The protein moiety composed of 17 amino acids[9]. An anticomplementary polysaccharide has been isolated from this fungus[10].

Agaricus xanthoderma

Two pigments - agaricon and xanthodermin were isolated from the extracts of fresh *A. xanthoderma*. These pigments exhibited antibacterial activity against *Bacillus subtilis* and *B. brevis*[11].



Agaricon



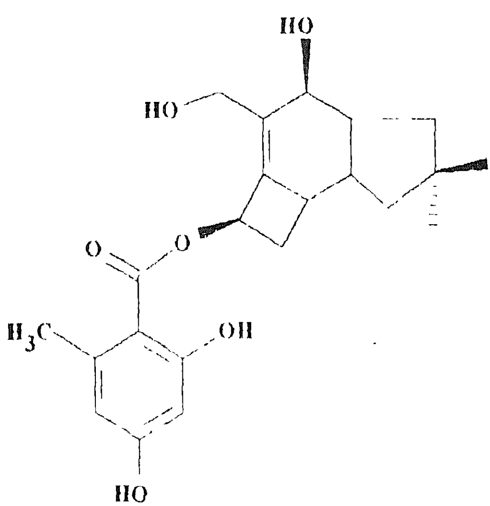
Xanthodermine

Agrocybe aegerita

Agrocybe aegerita Submerged fermentation of a malt wort or synthetic glucose medium with *A. aegerita* for 7-8 days with shaking gave a product which have bactericidal and bacteriostatic activity against 23 pathogenic bacteria and yeasts. The filtered medium (0.1 ml) had antibacterial and antifungal activity corresponding to 100 IU of streptomycin and 500 IU of fungicidin respectively[12].

Armillaria mellea

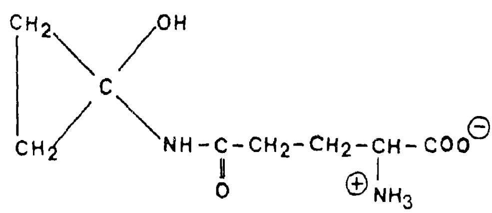
In China tablets containing artificially cultured mycelium of *A. mellea* (Tricholomataceae) are used for the treatment of dizziness, headache, neurasthenia, insomnia, numbness in lymphs and infantile convulsion[13]. Armillarin and armillaridin were isolated from the artificially cultured mycelium of *A. mellea*[14]. Armillyl orsilanate isolated from this fungus showed antibacterial activity[15].



Armillyl orsilanate

Coprinus

Several members of the genus *Coprinus* are edible. *C. comatus* and *C. atramentarius* are considered as delicacies and consumed in large quantities. Coprine, isolated from *C. atramentarius* shows disulfiram like activity[16].



Coprine

An immunoreactive protein with a m.wt. of 27,800 Da, which is mainly present in the cap of young basidiocarp was purified from the basidiomycete. *C. cinereus*[17].

Coprinus comatus

It is said that the consumption of certain mushrooms lowers blood glucose concentrations and may benefit the treatment of diabetes mellitus[18,19]. In mice fed with fruit bodies (3.6 g/kg body wt.) of *C. comatus* marginal lowering of plasma glucose was observed 10h after administration. The results suggest a slowly generated, mild hypoglycaemic effect of *C. comatus* in normal mice, accompanied by metabolic effects capable of interrupting body weight gain[20].

Coriolus spp.

A pharmaceutical preparation obtained by extraction of basidiomycetes mycelles of a *Coriolus* sp. has a glycoprotein as its active ingredient. The glycoprotein contains 80-83% protein and an elemental composition of 40.5% C, 6.2% H and 5.8% N. The active substance showed a number of pharmacological activities such as antihypertensive, antidiabetic, antithrombotic, blood platelet inhibiting, analgesic, antiinflammatory, vasodilatory etc[21].

Coriolus versicolor

Macromolecules isolated from the fruit bodies and mycelia of *C. versicolor* were found to possess antitumour and immunostimulant properties. From the capraphores of this fungus, a polysaccharide fraction, built up of glucose (96.44%), xylose (2.16%) and mannose (1.73%), showing antitumour activity was isolated[22].

PSP, a glycopeptide possessing antitumour and immunostimulant activities was obtained from the ethanol extract of *C. versicolor* mycelium[23], PSP (10-1000 μg/ml) induced the formation of interferon α and γ by human peripheral leucocytes. Moreover, PSP increased the phagocyte formation of host RE system. The results suggested that the antitumour effects of PSP may be related to its potentiation of host immune responses[24].

Flammulina velutipes

This edible mushroom is found in Kashmir Himalayas. Proflamin, an antitumour glycoprotein isolated from this fungus had been the subject of investigation for a long time. Recently a polysaccharide, PA3 DE with average m.wt. 5.4×10^6, isolated from this fungus had been shown to possess inhibitory activity against implanted Sarcoma-180 (Solid tumour) in mice[25].

Fomes japonicus

An antiteratogenic glycoprotein with average m.wt 1×10^4-1×10^6 was isolated from the basidiomycetes of this polyporous fungus[26].

Ganoderma lucidum

This polyporous fungus is an ingredient of several Chinese medicinal preparations. In China this drug is known as *Lin Zhi Cao*. *G. lucidum* is used in several medicinal preparations by the tribals of Madhya Pradesh in India. It is reputed to possess hypocholesterolemic activity. This fungus is rich in oxygenated lanosterol derived triterpenoids and more than 90 terpenoid compounds have been isolated from this fungus. Many of the triterpenoids isolated from *G. lucidum* are C-3 epimers and C-3/C-15 positional isomers in pairs.

Several of these triterpenoids were inhibitors of cholesterol biosynthesis. Bioassay of hypolipidemic activity was based on the inhibition of [2-MC] acetate incorporation into cholesterol by rat liver slices[27]. Biological activity was observed in the glycoproteins and polysaccharides obtained from the fruit bodies and mycelia of *G. lucidum*. An alkali soluble glycoprotein isolated from this fungus showed anticomplementary activity and it was also shown to increase haemolytic plate forming cells of the spleen in ICR mice[28]. A β-D-glucan isolated from this fungus showed immunostimulative antitumour activity[5]. An immunosuppressant glycoprotein having m.wt. 16000-18000, free of human hemagglutination ability is prepared from mycelia of *G. lucidum*. This glycoprotein is useful in the treatment of allergies[29]. Moderate hypoglycaemic activity was reported in the heteroglycan fraction obtained from this fungus[30]. A novel protein with mitogenic activity *in vitro* and immunomodulatory activity *in vivo* has been isolated from the mycelial

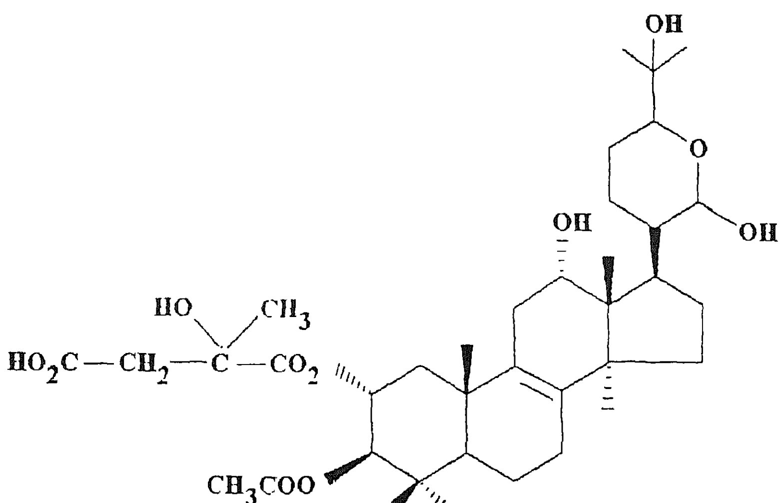
extracts of this medicinal fungus - Ling Zhi (*G. lucidum*) and has been named LZ-8[31].

A glycoprotein fraction GL isolated from the hot water soluble components of the basidiocarps of *G. lucidum* showed 81% inhibition of tumour growth in mice. This fraction had mol. wt. ~47KD and analysed for 82% polysaccharide, 8% protein and 0.9% hexosaminose. The polysaccharide moiety consisted of 63% glucose, 27% galactose, 7% mannose and 3% fucose. GL exerted the antitumour activity through immunopotentiation and not through direct cytotoxicity against the tumour[32].

From the cultured mycelium of *G. lucidum* Toth *et al.*, isolated ganoderic acids Z, Y, X, W, V and U were cytotoxic to hepatoma cells *in vitro*[33,34].

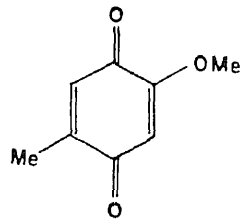
Hebeloma sinapizans

A cytotoxic lanostane triterpene namely 3- β-acetyl-2-α-(3-hydroxy-3′-methyl)glutaryl crustulinol was isolated from *H. sinapizans*[35].



Lentinus adhaerens

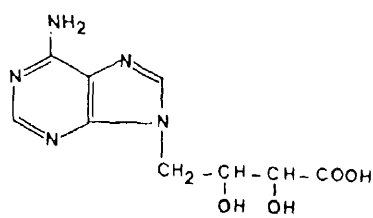
Another medicinal mushroom that has been studied from the genus Lentinus is L. adhaerens. 2-Methoxy-5-methyl-1,4- benzoquinone, a thromboxane A_2 receptor antagonist was isolated from the mycelial cultures of L. adhaerens. The compound



inhibits the U 46619 - induced aggregation of human blood platelets with an IC_{50} of 2.5 µg/ml (16.45µM) and is a new thromboxane A_2 receptor antagonist[36].

Lentinus edodes

L. edodes, commonly known as Shiitake has been credited with antitumour, antiviral, hypolipidemic, hypocholesterolemic and antibiotic properties. The antitumour properties are attributed to the polysaccharides-lentinan[37] and emitanin[38]. Lentinan is now used as an antitumour drug.

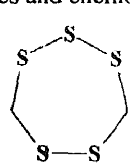


D-Eritadenine

The hypolipidemic property of this mushroom has been found to be due to the compound 2(R), 3(R)-dihydroxy-4-(9-adenyl)butyric acid, known as eritadenine[39].

Lenthionine, a 1,2,3,5,6-pentathiepene isolated from this fungus shows strong antibiotic activity[40,41].

Viral infection like AIDS and Immuno Depression Disease (IDD) are not responsive to existing antibiotics and chemotherapeutic agents like Azidothymid-



Lenthionine

ine (AZT) usually gives severe toxicities and side effects. In recent years, relatively nontoxic polysaccharides with immunostimulating properties have been found from natural sources like fungi and higher plants. In spite of the high complexity of the polysaccharides involved, certain structural similarities have been found for many of these immunostimulating compounds. These include the primary structure of the main chain not susceptible to hydrolysis by mammalian enzymes like polysaccharidases (e.g. α- and β-amylases), secondary structures stabilised by seven membered intramolecular hydrogen bonding and same exist as triple helical structures in solid state as revealed by X-ray analysis. Good correlations have been obtained between log activity and log molecular weight of lentinan isolated from L. edodes and its degraded products[42].

An antiviral composition, consisting of water soluble modified lignin containing sugars and proteins was extracted from *L. edodes* cultured in a medium consisting of materials derived from a plant containing lignin, was shown to prevent viral infections in both plants and animals[43]. A composition for treatment of AIDS was prepared from the mycelium of *L. edodes*. The preparation contained the sugars, fucose, galactose, mannose, xylose, arabinose, glucose and rhamnose and the inorganic elements K, Ca, Mn, Fe, Ni, Cu, Zn, Ge, Br, Rb and Sr[44]. A fraction, EPS4, obtained from *L. edodes* mycelium by ethanol precipitation followed by hydrophobic chromatography and gel filtration chromatography completely inhibited the HIV-1 induced cytopathic effect *in vitro* at concentration of > 10 g/ml. EPS4 is composed of water soluble lignins containing minor amounts of protein (3.2%) and sugars (12.2%) and exhibits *in vitro* immunostimulating and anti-HIV effect[45].

A polysaccharide isolated from *L. edodes* (25-50 mg/day orally or i.p.) was found to be effective in lowering the elevation of SGPT level in CCl_4, thioacetamide and prednisolone - intoxicated mice or rats. It was also found to increase the content of liver glycogen in CCl_4- intoxicated mice at 12.5 and 50 mg/kg/day[46].

LEM-HT a hot water extract prepared from the mycelium of *L. edodes*, contained sugars, proteins, P, S, Mg, Ca, K and Na. It analysed for sugars (44%), protein and others (31.4%). LEH-HT inhibited HIV infection of cultured human T-cells for 12 days at 0.5 μg/ml. LEM-HT was found to stimulate macrophages at 125 μg/ml and to stimulate production of interleukin[47].

Work on *L. edodes*, expanding over several years, is still going on and more interesting results are expected in the years to come.

Pleurotus ostreatus

P. ostreatus is an edible fungus and is cultivated in large quantities. A polysaccharide isolated from this fungus was found to lower serum and liver lipids in Syrian hamsters with hyperlipoproteinemia[48].

An antitumour glucan was isolated from the neutral polysaccharide fraction from the hot water extract of this mushroom. The glucan showed marked antitumour activity at a dose of 0.1 mg/kg. It is a highly branched (1Ä3)β-glucan having an average structure represented by a pentasaccharide segment consisting of one non-reducing terminal, one 3,6-di-O-substituted and 3,3-mono- O-substituted β-D-glucopyranosyl residues[49].

Pleuteus cervinus

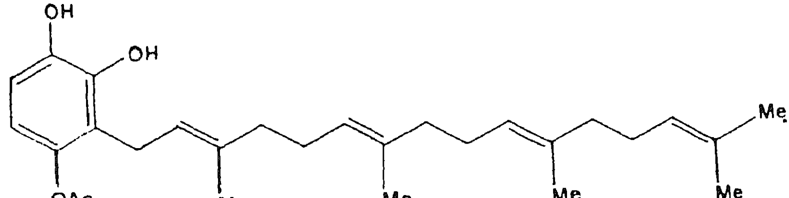
From the cultured mycelia of *P. cervinus*, a new antitumour constituent was isolated and named as Cervinan A. Cervinan A composed of a polysaccharide moiety (88%) and a protein moiety (1%). The polysaccharide moiety was a heteroglucan which consisted of glucose (92.4%), galactose (3.6%), mannose (4%) and traces of xylose and fucose. The protein moiety consisted of 17 amino acids[50].

Polyporus confluens

A polysaccharide fraction N-PS isolated from *P. confluens* cultivated in a defined medium showed antitumour activity. N-PS is a β-(1Ä6)-branched β-(1Ä3)-D-glucan[51]. Three xyloglucans were also isolated from this fungus which were shown to inhibit the growth of Sarcoma 180 cells in mice[52]. From the mycelium of cultured *P. confluens* a peptic ulcer inhibiting glycoprotein containing 90% sugars and 4.3% proteins was isolated. Oral administration of this glycoprotein markedly inhibited the HCl-EtOH induced peptic ulcer in rats[53].

Suillus granulatus

Suillin an antitumour principle isolated from *S. granulatus* was shown to be active against P-388 leukemia in *in vitro* and *in vivo* studies[54].

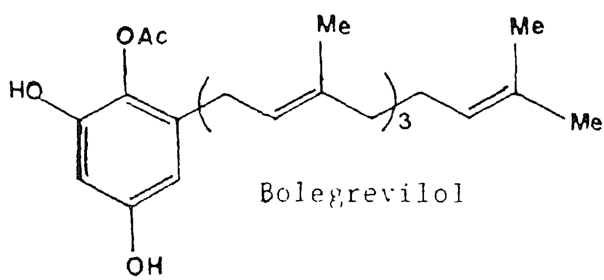


Suillin

1-Acetoxy-6-geranylgeranyl-2,4-dihydroxybenzene and 6-geranylgeranyl-2,4-dihydroxy-1-methoxybenzene isolated from this fungus was shown to possess antimicrobial properties[55].

Suillus grevillei

The compound bolegrevilol, isolated from the edible mushroom *S. grevillei* inhibited lipid peroxidation[30]. The structure of bolegrevilol was determined as 1-acetoxy-6- geranylgeranyl-2,4- dihydroxybenzene[56].



Bolegrevilol

Vitamin D in Fungi

Ergosterol is a common constituent in many of the mushrooms. Vitamin D content of edible mushrooms can be increased by exposure of the fruit bodies to solar or UV radiation. Exposure of the fruit bodies of *Lentinus edodes* to direct sunlight for several hours increased the Vitamin D content[57].

Acknowledgement

Authors are thankful to Western Ghat Development Programme, Department of Planning and Economic Affair (Western Ghat Cell), Govt. of Kerala (Grant. No. 6/94/plg.) for financial assistance.

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Cultivation and Utilization of ***Lentinus edodes*** – An Important Medicinal Mushroom

J.L.Kachroo

Regional Research Laboratory
Jammu

THE use of fungi for their medicinal values goes back to the antiquity. Since the earliest times mushrooms have been widely consumed not only for their taste, texture and nutritional consumption but also for their claimed therapeutic values. Mushrooms have noticeable place in folklore throughout the world and the tradition of many cultures past and present describe medicinal effect of variety of mushrooms. The most commonly cited potential medicinal application of higher edible fungus appears to be clavacin - a possible antitumour agent isolated from *Calvatia gigantica*, emerged indirectly from folklore, knowledge (Lucas, 1959). The fruit body of *Ganoderma lucidum* has been a popular folk or oriental medicine to cure various human diseases, such as hapatitis, hypertension, hyperchlostrolima and gastric cancer, and recent research has confirmed that extracts of *Ganoderma lucidum* can act as metablic regulators and effective remedies to treat number of diseases (Liu *et al.*, 1979, 1980; Chan 1986; Yang & Jong, 1987; Jong & Birmenghum, 1990, 1993). Studies in the last two decades conducted in Japan and United States have shown that cultivable mushrooms like *Agaricus bisporus*, (Mizuno *et al.*, 1992), *Lentinus edodes* and *Pleurotus Spp.* (Yashioka *et al.*, 1976), *Volvarella volvacea*, (Lin & Chou, 1984; Misaki *et al.*, 1986), *Flammulina velutipes* (Komasuka *et al.*, 1968, 1973) and *Tricholoma matsutaka* had some inhibitory effect on the growth of tumors.

Some mushroom extracts have also induced the formation of interferon, a defensive mechanism against some virus infections and have displayed hypo-cholestromic activity or an ability to lower cholestrol levels (Cochran, 1978).

According to Chang, (1993), the whole world production of mushroom for food is around 8 billion US dollars. The value of pharmaceutical and health products derived from mushroom is about 1 billion dollars. Much of the commercial interest in pharmaceuticals made from mushroom is centered in Japan, where products obtained from *Coriols vessicolor, Lentinus edodes* and *Schizophylum commune* are worth 800 million dollars each year (Chang, 1993). Other products derived from *Ganoderma* spp. are in use in Korea, Taiwan and China. The role of cultivable medicinal mushroom in the development and application of beneficial biological activities offers advantage that its active principle is safe and can be tolerated by human. Cultivating such mushroom would provide an adequate supply.

Lentinus edodes (Berle) Singer, the black forest mushroom known by its common name 'Shing - gu' in China and 'Shiitake' in Japan is well known edible fungus. It has long been cultivated extensively in low temperature area of China, Japan, Korea etc. Its cultivation originated in China during 1000 and 1100 AD (Chang & Miles 1987). Some authors believe that the cultivation technology developed in China was later on transferred into Japan, the major producer of *L. edodes* (Ito, 1978; Royse, 1985). Its historical record has been referred by (Chang & Miles 1987). In nature it grows as saprophyte on members of the family Fagaceae with Quercus species being the most colonized substrate.

Shiitake (*L. edodes*) has been found to have many attributes as healthy natural commodity. It is said to endew people with vigour and energy and is effective against hypertension and blood pressure. Recent experiments have shown shiitake stimulates the immune system which acts against cancer cells. It has been found to have antiviral as well as antitumour activities It is characterized by several substances i.e. gunosine-5, monophosphate, which induces nice flavour (Nakajume *et al.*,1961, Hauri *et al.*,1969) , aroma bearing "Canthionine" (Morita, & Kobayashi, 1966, 1967; Yarumoto *et al.*,1971, a,b; Vit D Fujita *et al.* 1969). Besides this it has additional culinary properties which are not available in other cultivable mushrooms.

Interest in the cultivation of Shiitake has recently been taken up by researchers in America, Germany, the Philippine, and many European Countries. The production of shiitake has gone up from 320,000 metric tonnes in 1986 to 402,000 metric tonnes in 1989-90 (Chang & Miles, 1991). In U.S.A. production has gone up from almost nothing in 1980 to 1.3 million kilograms in 1991-1992.

The nutrient contents of *L. edodes* reported by various workers have shown that it contains 17% of protein, 8% of fat; 67.5% of carbohydrate (Bano & Rajrathnum, 1982). It has been reported that shiitake is a good source of vitamins: Thiamine (7.8 mg), Riboflavin (7.9 mg), Niacin (54.9 mg) per 100 g of dried sample

(FAO 1972). The mineral constituents per 100 g of dried sample has been shown as Calcium (118 mg), Phosphorus, (650 mg) and Potash 1246 mg.

Cultivation

Traditionally the shiitake mushrooms have been cultivated on hardwood logs in natural environment, but this technique required more time for harvest, the supply used to be inconsistant as it used to be influnced by weather conditions. The awareness among the people for protection of forest and forest products diverted the attention of research workers to find an alternative technique of shiitake cultivation on other substrates like saw dust. The earliest commercial technique for the cultivation of shiitake on supplemented saw dust were developed in Japan, Taiwan and China (Isikowa, 1961; Ando, 1974; Mori *et al.*, 1974; Fuzisawa *et al.*, 1978a, 1978b; Fuzikowa & Hattori, 1979; Mee, 1978; Angles, 1979). This method has advantage that more mushrooms can be produced in less time than on logs, greater biological efficiency is attained and fresh market consistency maintained (Royx, 1985, 86; Royse *et al.*, 1986; Miller, Milles & Jong, 1987). In china and Taiwan cultivation is undertaken in an open, uncontrolled or semicontrolled environment, while in USA, Canada and Singapore cultivation is under environmental controlled conditions. The cultivation of shiitake on saw dust substrate is called synthetic log cullture. Farmers can make synthetic logs. The choice of cultivation depends on availability of finance and substrate. It can be a small business as with farmers in Japan and multi-million dollar industry involving sophiscated industrial technique.

Materials and Substrates

Many materials suitable for shiitake cultivation such as saw dust baggase, rice hulls, cotton seed hulls, corm cob, hay straw, shreded newspapers and other such materials have been used as the nutritive component of the substrate (Huang, 1987; Fuzisawa *et al.*, 1978 a,b). The substrates usually consists saw dust mixed with other supplements. Saw dust from different tree species can be used, production varies from saw dust of species to species, some giving favourable results where as others unfavourable growth and reproduction. Texture of saw dust has important role to play. The saw dust texture should be about 50% coarse saw dust and 50% fine grade for better aeration.

Supplements added to the saw dust includes carbon source, and a mixture of nitrogen source. A wide variety of starch viz rice bran, wheat bran, corn flour etc. can be used and for protein source soya meal etc. can be used. Tan and Chang (1989) reported that addition of 10% wheat flour resulted in doubling the yield. Research carried out at Regional Research Laboratory, Jammu (Anonymus, 1990) have shown increase in yield of shiitake with the addition of wheat bran. Tan and Chang (1987, 1989) while working with different supplements found that addition of used tea leaves increased the yield by 6-7 times, however, they did not find any increase

in yield with the addition of sucrose, tannic acid, and IAA to the substrate. Calcium carbonate and Calcium sulphate is used to increase the permeability.

A good variety of technique and formulation exists for the preparation of the substrate. The selection of satisfactory formulae of the enriched substrate depends upon the quality of saw dust and nutrient supplement which can be found by productivity test. A few formulae are reported here.

1.	Regional Research Laboratory (1990)	
	Saw dust popular	75 kg
	Rice bran	25 kg
	Calcium carbonate	1 kg
	Water added	60%
2.	Tan & Chang (1989)	
	Sawdust	76% -78%
	Wheat bran	5%
	Used tea leaves	17-19%
	Water	65%
3.	Tan and Chang (1989)	
	Sawdust	94%
	Wheat bran	5%
	Calcium	1%
	Water	66%
4.	Royse *et al.*	
	Sawdust	80%
	Wheat bran	10%
	Millet	10%
	Water	65%
5.	Han *et al.*	
	Hardwood sawdust	80.800 kg
	Rice bran	10 kg
	Calcium carbonate	20 g
	Water adjusted	60%
6.	Mori *et al.*	
	Sawdust Oak	80 kg
	Rice bran	20 kg
	Water content	65%

All the ingrediants are mixed thoroughly and the water added. The amount of water required varies with the type of sawdust and its capacity to absorb moisture. The correct water content is of peramount importance and should be between 55-68%, the *p*H should be around 5.5

(A) Packing the substrate in bags: The mixed substrate is packed in heat resistant polypropalene bags, the loose end of the bag is gathered around and fastened to a ring made from cut PVC pipe (5 cm in diameter and 2-3 cm long) or ready made rings plugged with cotton. The cotton plug should be covered with autoclavable plastic or with craft paper. Wide mouthed glass bottles or plastic bottles (heat reistant) can also be used. The size and thickness of the bags can vary. However, the research carried with the thickness of the bag by Chantarasnit (1989) has shown that thinner bags have higher rate of contamination possibly because they were more easily subjected to damage during sterilization and transportation. Therefore, thicker the bag better it is. The size and the shape of the bag gives shape to the synthetic log. Experiments conducted by Royse & Bahler (1989) have shown that productivity and mushroom size were significantly affected by log diameter. As synthetic log diameter increased biological efficiency decreased, while mushroom size increased. Bag size of 18×25 cm or 29×27 cm is the best.

(B) Sterilization/Pasteurization: The substrate after packing requires sterilization or pasteurization before use to kill unwanted competitive micro-organisms that may be harmful for growing shiitake. Heat treatment may be carried out in an autoclave at 121°C for 1 hour (Miles & Jong, 1987) or in a brick and cement lined tower at 90°C for 5-7 hours (Auetragul, 1984; Miles & Chang, 1987). Both systems of heat treatment are used commercially. Royse *et al.*,(1985) found reduced yield on sawdust that was heat treated but not autoclaved. Badham (1988) described heat that produced equal to that of an autoclove substrate. Chantarasnit (1989), while conducting trial with four methods of sterilization to overcome disease problem found single steaming and intermittent steaming better.

(C) Spawning/Inoculation: The substrate after heat treatment is cooled, and moved to the spawn room or inoculation room. Grain or sawdust spawn is commonly used for spawning the substrate in bags. Care must be taken during spawning procedure, since air borne micro-organisms can easily be introduced to the exposed substrate. The spawn should be thoroughly checked for any contaminants or any abnormal growth. Different strains of spawn are available and they have adaptation to different nutrient conditions and environment. Proper selection of strain should be done. For spawning two workers are needed, one will open the plug of the bag under flame and other worker will put spawn from the spawn bottles to the bags. Following precautions should be taken before spawning:

(i) Clean the inoculating room or chamber with soap solution or alcohol.

(ii) Workers should sterilize their hands and inoculating instruments.

(iii) Clean the spawn bottle with alcohol.

(iv) Flame the spawn bottles after everytime its mouth is opened.

(D) Spawn running/Incubation: The spawned bags are taken in a room where ambient temperature of 24°±2C is maintained, for the colonization of the substrate with the mycelium. For the proper colonization of substrate it may take 30-100

days. During this period the bags should be checked for spawn growth, as well as for contaminants. The bags are placed in shelves or racks one over the other. Care should be taken that bags do not get punctured as weed moulds can enter through the punctured points. Bags with suspected spawn growth should be discarded, as they will encourage the spread of disease, which may result in crop loss. To combat competition by weed moulds the floor of the spawn running room should be sprinkled with calcium carbonate. Mycelium must grow from the top of the substrate to the bottom for complete spawn run. Maintenance of substrate temperature within the range provides maximum growth and minimize the time required for complete spawn running. A constant temperature is better than the fluctuating temperature for mycelial growth. During spawn running the mycelium is white till it completely colonizes the substrate. Afterwards the colonized surface begins to turn brown and some water drops develop. A longer spawn run, usually results in higher rate of production during the cropping cycle (Royse, 1985; Rose & Bahtler, 1986). Spawn running takes place in darkness but a little light is also needed.

(E) Cropping/Fruiting: At the end of spawn running the plastic bags are transferred to the fruiting room. The substrate is exposed by stripping of the entire plastic covering of the bag. Vigorous watering is applied by fine mist overhead sprays to induce fruiting. In the beginning relative humidity of 90% should be there in the production room which should be reduced to 80% when fruit bodies start developing. Initiation of fruiting depends on light and is required for normal production of fruit body. Two hours of flourescent light is needed. Temperature required for fruiting is lesser than spawn running. A temperature range of 14-16°C is optimum for quality of mushroom. Higher temperatures have been reported to elongate the stipe length and pileus diameter (Tokimoto & Kamatru, 1978). Adequate air changes are required to remove CO_2. Fluctuation of temperature during fruiting has been found to have added advantage.

(F) Harvesting: After placement of the bags in the production room mushrooms will usually begin to appear after 7-10 days and should reach to maturity after 3-4 days of initiation. They must be harvested before they extend to maturity because quality wise matured fruit bodies are considered inferior. The fruit bodies should be cut off with the knife and not sniffed or twisted by hand to prevent the disruption of sawdust bags and avoiding the attack by insects and moulds. The harvested fruit bodies should be placed top down in the collection basket, bag or tray carefully, as careless handling reduces their commercial value. Under normal conditions 200-250 g of shiitake may be obtained per kg of the substrate. About four flushes of mushroom may be obtained. However, experimental results at Regional Research Labotatory and other places have shown that about 55-60% of the harvest is obtained in the first flush, 25-30% in the second flush, while the yield from 3-4 flush ranges from 8-10%

(G) Processing: After the shiitake are harvested they are sorted and classified according to quality. Mushroms are sold as fresh or in dried form. Mushrooms meant for fresh sale should be refrigerated and those meant for future sale should be dried. Dried mushrooms are convenient for long term storage, transportation and prevents deterioration. There are two methods of drying.

(i) Sun drying

(ii) Heat drying/Thermal heat drying

Drying should be carried out within 3-4 hours of harvesting.

Sun drying: The picked mushrooms are arranged in a single layer on shelves or on clean sheets and exposed to the sun. The time needed for sun drying varies depending upon the season. Usually it takes 3-4 days for drying.

Heat drying: Heat drying of shiitake mushrooms should be started with low temperature of 30°C and after 5-6 hours temperature should be raised slowly and kept at a temperature of 60°C for a period of 16-18 hours so that dried product does not have moisture more than 13%. Dried mushrooms should be packed in polythene bags, sealed and kept at safe cool and dark place.

Diseases, Pests and Control

Many pests and pathogens affect the yield and quality of mushrooms. The disorder caused by them are noticed during various stages of its cultivation. Basically farm hygiene at every stage of mushroom cultivation is the best approach to the control of these maladies in the farm. One of the production problem in shiitake cultivation is its competition with other fungi, bacteria and insects. These weed fungi compete for available nutrients and produce metabolites that inhibit the growth of shiitake mycelium (Tokimoto, 1985; Badham,1991, 1988; Wert,1983; Pryzbylowiez & Dongohu, 1988). The group of fungi causing disease are *Alternaria* spp., *Aspergillus* spp., *Epicocum* spp., *Pencillium* spp., and different species of *Trichoderma* Control of fungal diseases is best achieved by careful farm management and hygiene. In order to prevent the attack of moulds following points should be taken care of during cultivation:

(i) Select proper substrate and supplement for preparation of the synthetic logs (Disease free & fresh).

(ii) Adjust the required *p*H and moisture of the substrate.

(iii) Follow proper sterilization or pasteurization time.

(iv) Inoculate substrate in more aseptic way, observe complete cleanliness.

(v) While transporting the bags, take care that bags do not get punctured.

(vi) Eradicate infected block during spawn running, fruiting etc.

(vii) Maintain good hygiene around uncolonized substrate.

(vii) Minimize water on colonized block.

(ix) Use disease free spawn.

Besides fungi, bacteria also infect the shiitake cultivation. Miller & Jong (1987) has reported occurrence of anaerobic bacterium developing in bag cultures. These bacteria are heat resistant and difficult to control. In some cases bacteria will prevent the growth and in other cases it inhibits the growth. In our experimental trial at Regional Research Laboratory we found mother culture spawn responsible for the development of bacterial disease. It inhibited the mycelial growth and developed wet patches in the substrate. The main method of control is to ensure bacterial free source of saw dust, suppliments and spawn and follow the measures suggested to avoid moulds.

Insects: Little information is available about the insects that effect the synthetic beds of shiitake. Mites, sciarid fly and springtail are the insects which appear during the course of cultivation. They appear on bags with weed moulds. The most common method of control is to remove infected bags. Application of insecticides on the bags is not recommended. Dust the floor of the growing room with B.H.C. or lime and apply DDVP aerosal inside the cultivation room.

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Airborne Fungal Spores and Diseases of Medicinal Plants

A. K. Jain

School of Studies in Botany
Jiwaji University
Gwalior - 474 01

EVERSINCE the appearance of man on this earth, man depended on plants for the sustenance of his life and treating the diseases. The earliest literature of various civilizations of the world have the mention of several medicinal plants for curing various diseases. The knowledge regarding multifarious uses of plants developed with the advance of civilization. People came to know about more and more uses of plants through trial and error. Every epoch of civilization has left its landmark and produced a distinct culture regarding this natural science. Thousands of medicinal plants have been mentioned in various Indian literature. Ayurveda is supposed to be the science of medicine and has its roots in Indian culture. Right from the vedic period down to the present day, Ayurveda has remained an intimate part of our daily life.

Now-a-days herbal medicines are widely used by ayurvedic practitioners, pharmaceuticals, tribal medicine men, herbal vendors and other people of the society. Due to various side and adverse effects of allopathic medicines, more emphasis has been laid on the use of herbal drugs.

Impact of Microorganisms

Aerobiological studies indicate that a large number of microorganisms like fungal spores, bacteria, viruses etc. are present in the air throughout the year in fluctuating amount. Various climatic factors play an important role in the dispersal of such

organisms from their sources. Various plant parts like root, stem, bark, leaves, flowers, fruits etc. are used in making drugs. Plants or their parts are collected from forests or cultivated fields and stored in houses or godowns.

The drugs are given to patients either in crude form or processed, depending on the ailment. Unlike allopathic drugs the medicinal plants are not preserved or stored under hygienic or clean environment. In forests, fields or other natural habitats also fungal spores or other microorganisms attack plants and cause various diseases.

Ayurvedic drugs consist of various components like cellulose, resins, gums, terpenes, sugars, alkaloids, starch and other substances. All these serve as substrates for various microorganisms which are predominant in air, soil, water etc. These microorganisms contaminate medicinal plants/drugs during harvesting, transporting, processing or storing and cause various diseases (Table 1). The maintenance of sterility is extremely essential in case of storage and preservation of medicinal

Table 1 Diseases of some common medicinal plants and their causal organisms.

Medicinal plant	Affected Part/Disease	Causal organisms
Ailanthus excelsa	Bark and leaves	*Didymosphaeria* sp.
Amaranthus viridis	White rust	*Albugo bliti*
Albizzia lebbeck	Bark	*Hypoxylon* sp.
Cannabis sativa	Wilt, Leaf Spot	*Fusarium* sp , *Corticum solani*
Carum carvi	Entire plant	*Aspergillus niger*
Catharanthus rosea	Blight	*Phytophthora* sp.
Coriander sp.		*Rhizopus nigricans*
Crotolaria sp	Wilt	*Fusarium udum*
	Stem, Rot	*Corticum solani*
Cuminum sp	Blight	*Alternaria* sp.
Curcuma longa	Rot of Rhizomes	*Sclerotium rolfsii*
Cyperus spp	Bulb	*Duosporium* sp.
	Powdery Mildew	*Erysiphe* sp.
Datura spp.	Wilt	*Sclerotium rolfsii*
	Root Rot	*Sclerotium rolfsii*
	Leaf Spot	*Alternaria* sp.
Embelia ribes	Seeds	*Aspergillus niger*
Eucalyptus sp	Leaf Spot	*Cylindrocladium* sp.
Emblica officinalis	Fruit Rot	*Penicillium* sp.

(Table 1 Contd.)

Ficus carica	Rust	*Cerotelium fici*
Ficus benghalensis	Leaves	*Exosporium* sp.
Gymnema sylvestre	Branches	*Sillia* sp.
Hyoscyamus niger	Leaf Spot	*Ascochyta* sp.
	Root Rot	*Thielvia basicola*
	Mildew	*Pernospora hyoscymi*
Indigofera spp.	Leaves	*Parodiella* sp.
Jasminum spp.	Leaf blight	*Cercospora jasminicola*
	Rust	*Uremyces* sp.
	Wilt	*Sclerotium rolfsii*
Mentha spp.	Powdery mildew	*Erysiphe* sp.
	Stolon Rot	*Rhizoctonia* sp.
	Sclerotium Rot	*Sclerotium* sp.
	Rust	*Puccinia menthae*
Ocimum basillicum	Leaf spot	*Cercospora* sp.
Pongamia glabra	Stem, Leaves	*Triblidaria* sp.
Ricinus communis	Rust	*Melampsora ricini*
	Leaf spot	*Cercospora ricinella*
Rauvolfia serpentina	Wilt	*Fusarium* sp.
	Leaf blight	*Alternaria tenuis*
	Leaf spot	*Cercospora* sp.
Solanum nigrum	Leaf spot	*Cercospora* sp.
Solanum aviculare	Wilt	*Fusarium* sp.
Strychnos nuxvomica	Fruit Rot	*Cladosporium* sp.
Tamarindus indicus	Leaf spot	*Exosporium* sp.
Tephrosia spp.	Wilt and Stem burn	*Pythium delense*
Terminalia chebula	Fruit Rot	*Aspergillus* sp.
Triphla churna		*Aspergillus niger*

plants. Consumption of such contaminated drugs is not only ineffective but also invites risks of chronic sickness.

The infection causes loss of originality and reduces market value of the drug. Several biochemical changes cause loss of active principle and many a times produce toxic substances which are harmful to human beings and animals (Rao & Shukla, 1994). Extensive work in this field has also been made at different places by various workers (Chattopadhyaya, 1961; Sastry, 1969; Misra & Kamal, 1972; Sharma& Jain, 1977; Sastry *et al.*, 1982 and Bhide & Risbud, 1983).

Aerobiological Investigations

A regular aero-survey of natural habitats and store houses of medicinal plants provides the complete picture of various airborne microbes. Studies on sources, dispersal and impact of various such organisms could play a key role in preventing the mass deterioration and maintaining the quality of drugs.

In the present study attempt has been made to find out the incidence of airborne fungal spores observed around various medicinal plants.

Aerobiological investigations were made at and around open fields, natural forests and in store houses of medicinal plants. A regular exposure of petriplates with PDA (Potato Dextrose Agar) media was made for one year. After incubation, fungal colonies which appeared on media were studies and identified. Air-sampler (Jain and Misra, 1988) was also used to trap fungal spores. Two glycerine jelly coated slides were fixed in the air-sampler and replaced by fresh slides after 24 hours. Fungal spores, trapped over the slide surface were identified and percentage value calculated.

A comparative account of mycoflora observed at some other places (Table 2) has been made. These studies have been made at Lucknow (Mittre and Khandelwal, 1973), Aurangabad (Tilak, 1982), Bangalore (Agashe *et al.*, 1983), Delhi (Singh Gangal, 1986), Calcutta (Nandi & Chanda, 1989) and Guwahati (Sarma & Sarma, 1993).

Analysis of Data

A large number of medicinal plants occur wild in forest areas and other localities while several others are cultivated by various techniques. Many plants/parts are transported from one to other places and stored in godowns, either packed or unpacked. The study reveals that various fungal spores types are active under both conditions i.e., outdoor or indoor. Many seed-borne or soil-borne diseases have their root causes in air-borne pathogens. Several such fungal spores are common at all localities and include species of *Alternaria, Aspergillus, Cladosporium, Curvularia, Epicoccum, Helminthosporium, Nigrospora, Periconia* etc. Many spore types which were not recorded also cause serious hazards to medicinal plants.

Some fungi like species of *Alternaria, Fusarium, Drechslera, Curvularia, Phoma* etc. proliferate under high moisture content of stored herbal parts like fruits, seeds, rhizomes etc. Once the tissues get infected these fungi continue to proliferate further even under such storage conditions. Certain physical factors like impurities, improper packing, are also responsible for invasion of fungal spores.

Continuous aerobiological survey of any locality could certainly provide a clear picture about seasonal dispersal of such organisms. Forecasting about occurrence of diseases can be made by preparing spore calendar. Spray of fungicides or

Table 2 - Incidence of air-borne fungal spores observed at some important places in India

Spore Type	Different localities and incidence of fungal spores (%)						
	Gwalior	Lucknow	Calcutta	Delhi	Bangalore	Guwahati	Aurangabad
1	2	3	4	5	6	7	8
Alternaria	52.76	35.8	4.5	41.7	19.97	8.75	5.4
Aspergillus	4.34	6.7	32.7	12.3	-	-	0.9
Acrothecium	-	0.4	-	1.1	-	-	-
Bipolaris	2.13	-	-	-	3.24	-	-
Bispora	2.22	-	-	-	-	-	-
Botryodiploidia	-	-	0.5	-	-	-	0.09
Botrytis	0.23	-	-	-	-	-	-
Cercospora	0.14	3.4	0.5	-	-	-	-
Cladosporium	2.71	5.0	8.8	28.5	51.73	21.00	31.31
Chaetomium	0.23	1.04	31.7	-	-	-	-
Coprinus	-	-	1.3	-	-	-	-
Corynospora	0.23	-	-	-	-	-	-
Chaetomella	-	-	-	-	-	1.01	-
Coniothecium	-	-	-	-	-	-	0.01
Curvularia	4.25	0.9	0.5	3.4	5.24	18.5	4.4
Diplodia	-	0.45	-	-	-	-	0.6
Drechslera	2.04	-	3.8	-	-	9.88	0.42

(Table 2 Contd).

1	2	3	4	5	6	7	8
Dendrographium	-	-	0.7	-	-	-	-
Epicoccum	0.86	0.89	-	5.4	-	2.50	0.25
Fusariella	0.36	-	-	-	-	-	0.24
Fusarium	0.09	0.8	4.9	-	-	8.75	-
Gonoderma	-	-	-	-	-	-	-
Haplospora	0.36	-	-	-	-	-	-
Haplosporella	0.77	-	-	-	-	-	0.6
Helminthosporium	4.30	12.8	0.5	-	1.08	-	3.15
Hirudinaria	0.23	-	-	-	-	-	0.01
Monilia	0.45	-	-	-	-	-	-
Namnonella	1.63	-	-	-	-	-	-
Neurospora	-	-	-	-	-	0.95	-
Nigrospora	4.9	3.4	6.8	-	0.66	4.85	4.3
Oidia	-	-	-	-	-	-	-
Papularia	-	-	0.5	-	-	-	-
Periconia	0.18	-	2.2	-	1.42	0.68	3.35
Pithomyces	0.27	-	1.1	-	-	-	1.00
Penicillium	-	-	-	-	-	-	0.51
Phoma	-	-	-	-	-	-	0.04
Pleospora	-	-	-	-	-	-	-

(Table 2 Contd).

1	2	3	4	5	6	7	8
Pyricularia	0.77	-	-	-	-	-	0.33
Rhizopus	0.14	-	-	-	-	-	-
Sclerotium	-	-	-	-	-	-	0.6
Smut spores	5.29	1 4	-	4.8	-	1.51	-
Speqazzinia	-	-	0.5	-	-	-	0.25
Sphaeropsis	2.99	-	-	-	-	-	0.02
Teleuto spores (*Puccinia*)	0.05	0.05	-	-	-	-	-
Tetraploa	0 59	0.2	-	-	-	-	0.12
Thielaviopsis	0.41	-	-	-	-	-	-
Tilletia	0.08	-	-	-	-	-	-
Torula	0 09	-	13.1	-	-	1.05	1.8
Trichoderma	0.05	-	-	-	-	1.08	0.56
Trichothecium	0.05	-	-	-	-	-	0.04
Trichoconis	-	-	-	-	0.72	1.01	0.03
Uredospores (*Puccinia*)	5.93	12.1	-	-	1.1	-	-
Valsaria	0.41	-	-	-	-	-	-
Veutura	-	-	-	-	-	-	-
Zygodesmus	-	-	-	-	-	-	0.44
Unidentified	2.31	2 0	21.5	2.8	-	2.25	-

other chemicals could be done well in advance to save medicinal plants of fields, forests or storages.

Acknowledgements

The author is grateful to Prof. R.S.Das, Head, School of Studies in Botany, Jiwaji University, Gwalior for valuable suggestions and to ICMR, New Delhi for providing financial assistance.

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